98/81754 PCT/EP 99/08744

Europäisches Patentamt | European Patent Office | Office européen des brevets

EJU

REC'D 17 JAN 2000
WIPO PCT

EP 98/8744

Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

98121478.6

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts:
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

I.L.C. HATTEN-HECKMAN

DEN HAAG, DEN
THE HAGUE, 11/01/00
LA HAYE, LE

THIS PAGE BLANK (USPTO)

Europäisches Patentamt
European Patent Office
Office européen des brevets

Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

Anmeldung Nr.:
Application no.:
Demande n°: 98121478.6

Anmeldetag:
Date of filing:
Date de dépôt: 12/11/98

Anmelder:
Applicant(s):
Demandeur(s):
Nitsch, Roger M., Prof. Dr.
20146 Hamburg
GERMANY

Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:
Methods of diagnosing or treating neurological diseases

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat: State: Pays:	Tag: Date: Date:	Aktenzeichen: File no. Numéro de dépôt:

Internationale Patentklassifikation:
International Patent classification:
Classification internationale des brevets:
C12N15/12, C07K14/47, C12Q1/68, G01N33/68, A61K38/17

Am Anmeldetag benannte Vertragstaaten:
Contracting states designated at date of filing: AT/BE/CH/CY/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE
Etats contractants désignés lors du dépôt:

Bemerkungen:
Remarks:
Remarques:

THIS PAGE BLANK (USPTO)

METHODS OF DIAGNOSING OR TREATING NEUROLOGICAL DISEASES

Neurological diseases are widely spread within a population and have a strong impact not only on patients' life but also on society as such. Therefore, there is a strong need to elucidate the causes and the underlying pathogenesis of such neurological diseases. Among such neurological diseases, Alzheimer's disease (AD) has a predominant position. Alzheimer's disease, first described by the Bavarian psychiatrist Alois Alzheimer in 1907, is a progressive neurological disorder which begins with short term memory loss and proceeds to loss of cognitive functions, disorientation, impairment of judgment and reasoning and, ultimately, dementia. It is the most common cause of dementia. The neuropathology is characterized by the formation in brain of amyloid plaques and neurofibrillary tangles. AD has been estimated to afflict 5 to 11 percent of the population over age 65 and as much as 47 percent of the population over age 85. Moreover, as adults, born during the population boom of the 1940's and 1950's, approach the age when AD becomes more prevalent, the control and treatment of AD will become an even more significant health care problem. Familial forms of AD are genetically heterogeneous, but most with early onset are linked to mutations in the presenilin genes *PSEN1* and *PSEN2*, as well as to mutations of the amyloid precursor gene *APP*. The majority of AD patients have no obvious family history and are classified as sporadic AD.

As neurological diseases, in particular AD, are a growing social and medical problem, there is a strong need for methods of diagnosing or prognosing said diseases in subjects as well as for methods of treatment.

In one aspect, the invention features a protein molecule (hereinafter "SELADIN-1") shown in SEQ ID NO. 1.

In another aspect, the invention features an isolated nucleic acid molecule encoding SELADIN-1 shown in SEQ ID NO. 1. Said nucleic acid molecule can be a DNA molecule, such as a genomic DNA molecule or a cDNA molecule, or an RNA molecule, such as a mRNA molecule. In particular, said nucleic acid molecule can be a cDNA

molecule having a coding sequence substantially the same as the coding sequence of SEQ ID NO. 2.

In another aspect, the invention features an oligonucleotide which hybridizes with a base sequence encoding SELADIN-1 shown in SEQ ID NO.1 in a solution of 0.2xSSC (standard saline citrate) and 0.1 % SDS at 60 °C.

In another aspect, the invention features an isolated DNA comprising a base sequence which hybridizes with said base sequence encoding SELADIN-1 shown in SEQ ID NO. 1 in a solution of 50 % formamide, 4xSSC, 50 mM HEPES, pH 7.0, 10x Denhardt's solution, 100 µg/ml thermally denatured salmon sperm DNA at 42 °C. The invention further aims at an oligonucleotide which hybridizes with a base sequence comprising the afore mentioned DNA molecule in a solution of 0.2xSSC and 0.1 % SDS at 60 °C.

"Variants" of a protein molecule shown in SEQ ID NO.1 include e.g. proteins with conservative amino acid substitutions in highly conservative regions. For example, isoleucine, valine and leucine can each be substituted for one another. Aspartate and glutamate can be substituted for each other. Glutamine and asparagine can be substituted for each other. Serine and threonine can be substituted for each other. Amino acid substitutions in less conservative regions include e.g.: Isoleucine, valine and leucine can each be substituted for one another. Aspartate and glutamate can be substituted for each other. Glutamine and asparagine can be substituted for each other. Serine and threonine can be substituted for each other. Glycine and alanine can be substituted for each other. Alanine and valine can be substituted for each other. Methionine can be substituted for each of leucine, isoleucine or valine, and vice versa. Lysine and arginine can be substituted for each other. One of aspartate and glutamate can be substituted for one of arginine or lysine, and vice versa. Histidine can be substituted for arginine or lysine, and vice versa. Glutamine and glutamate can be substituted for each other. Asparagine and aspartate can be substituted for each other.

"Variants" of SELADIN-1 shown in SEQ ID NO.1 also include .g. protein molecules which are encoded by DNA fragments comprising base sequences which hybridiz with

said base sequence encoding SELADIN-1 shown in SEQ ID NO. 1 in a solution of 50 % formamide, 4xSSC, 50 mM HEPES, pH 7.0, 10x Denhardt's solution, 100 µg/ml thermally denatured salmon sperm DNA at 42 °C.

The invention further features an antibody specifically immunoreactive with an immunogen, wherein said immunogen is SELADIN-1 shown in SEQ ID NO. 1 or a variant or fragment thereof. In another aspect, the invention aims at a method of detecting pathological structures in the brain of a subject which comprises immunocytochemically staining said pathological structures with said antibody.

In another aspect, the invention features a vector comprising the isolated nucleic acid encoding a SELADIN-1 shown in SEQ ID NO. 1 or a variant thereof. In preferred embodiments, a virus, a bacteriophage, or a plasmid comprises said nucleic acid.

In still another aspect, the invention features a plasmid adapted for expression in a bacterial cell which comprises a nucleic acid molecule encoding SELADIN-1 shown in SEQ ID NO. 1 or a variant thereof, and the regulatory elements necessary for expression of said molecule in the bacterial cell.

In a further aspect, the invention features a plasmid adapted for expression in a yeast cell which comprises a nucleic acid molecule encoding SELADIN-1 shown in SEQ ID NO. 1 or a variant thereof, and the regulatory elements necessary for expression of said molecule in the yeast cell.

In another aspect, the invention features a plasmid adapted for expression in a mammalian cell which comprises a nucleic acid molecule encoding SELADIN-1 shown in SEQ ID NO.1 or a variant thereof, and the regulatory elements necessary for expression of said molecule in the mammalian cell.

In a further aspect, the invention features a cell transformed with a nucleic acid molecule encoding SELADIN-1 shown in SEQ ID NO. 1 or a variant thereof. In preferred

mbodiments, said cell is a bacterial cell, a yeast cell, a mammalian cell, or an insect cell.

In a further aspect, the invention features a bacterial cell comprising a plasmid adapted for expression in a bacterial cell, said plasmid comprises a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, and the regulatory elements necessary for expression of said molecule in the bacterial cell.

In a further aspect, the invention features a yeast cell comprising a plasmid adapted for expression in a yeast cell, said plasmid comprises a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, and the regulatory elements necessary for expression of said molecule in the yeast cell.

In another aspect, the invention features a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, said plasmid comprises a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO.1, and the regulatory elements necessary for expression of said molecule in the mammalian cell.

In preferred embodiments, said subjects suffer from Alzheimer's disease and related neurofibrillary disorders, or neurodegenerative states characterized by cell degeneration or cell death. Further examples of neurological diseases are Parkinson´s disease, Huntington disease, Amyotrophic lateralsclerosis, Pick´s disease.

In another aspect, the invention features a method of diagnosing or prognosing a neurological disease in a subject comprising:
determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of
a) a protein molecule shown in SEQ ID NO. 1,
b) a variant or fragment thereof,
c) a nucleic acid molecule encoding a prot in molecule shown in SEQ ID NO. 1, a variant or fragment th reof,

d) a molecule affecting directly or indirectly a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to c),

) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c),

in a sample from said subject,

and

comparing said level, or said activity, or both said level and said activity, of at least one of said substances a) to e) to a reference value representing a known disease or health status,

thereby diagnosing or prognosing said neurological disease in said subject.

In preferred embodiments, said subjects suffer from Alzheimer's disease and related neurofibrillary disorders, or neurodegenerative states characterized by cell degeneration or cell death. Further examples of neurological diseases are Parkinson´s disease, Huntington disease, Amyotrophic lateralsclerosis, Pick´s disease.

It is particularly preferred that said sample is a brain tissue or other body cells including blood cells and skin fibroblasts. According to the present invention, a reduction in the level, or activity, or both said level and said activity, of SELADIN-1 or *SELADIN-1* transcripts in said subject's brain regions affected heavily by neurodegeneration relative to a reference value indicates a diagnosis or prognosis of Alzheimer's disease. Predominantly neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus and the amygdala degenerate in Alzheimer's disease.

It is also particularly preferred that said sample is cerebrospinal fluid or another body fluid.

It might be preferred that said subject has previously been determined to have one or more factors indicating that such subject is afflicted with a neurological disease.

In preferred embodiments, said subject can be a human, an experimental animal, e.g. a rat or a mouse, a domestic animal, or a non-human primate, e.g. a monkey. The experimental animal can be an animal model for a disorder, e.g. a transgenic mouse with an Alzheimer's-type neuropathology.

In preferred embodiments, at least one of said substances is detected using an immunoassay, an enzyme activity assay and/or a binding assay.

In preferred embodiments, measurement of the level of transcription products of the *SELADIN-1* gene, or a variant thereof, is performed in body cells using Northern blots with probes specific for the *SELADIN-1* gene or said variant. Quantitative PCR with primer combinations to amplify *SELADIN-1* gene-specific sequences from cDNA obtained by reverse transcription of RNA extracted from body cells of a subject can also be applied. These techniques are known to those of ordinary skill in the art (see e.g. Watson et al., Rekombinierte DNA, 2nd edition, Spektrum Akademischer Verlag GmbH, Heidelberg, 1993; Watson et al., Recombinant DNA, 2nd ed. W.H. Freeman and Company, 1992).

In preferred embodiments, said level or activity of the protein molecule shown in SEQ ID NO. 1, or a variant or fragment thereof, is detected using an immunoassay. These assays can measure the amount of binding between said protein molecule ("SELADIN-1") and an anti-SELADIN-1 antibody by the use of enzymatic, chromodynamic, radioactive, or luminescent labels which are attached to either the anti-SELADIN-1 antibody or a secondary antibody which binds the anti-SELADIN-1 antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow et al., Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

The antibody or ligand to be used should preferably specifically detect SELADIN-1 or a variant or fragment thereof. It is preferred that it does not substantially interact with any other protein present in said sample.

Monoclonal antibodies capable of recognizing a protein molecule of SEQ ID NO. 1 or a variant or fragment thereof can be prepared using methods known in the art (see e.g. Köhler and Milstein, Nature 256, 495 - 497 1975; Kozbor et al., Immunol. Today 4, 72, 1983; Cole et al., Monoclonal antibodies and cancer therapy, Alan R. Liss, Inc., pp 77 - 96, 1985; Marks et al., J. Biol. Chem., 16007 - 16010, 1992; the contents of which are incorporated herein by reference). Such monoclonal antibodies or fragments thereof can also be produced by alternative methods known to those of skill in the art of recombinant DNA technology (see e.g. Sastry et al, PNAS 86: 5728, 1989; ; Watson et al., Rekombinierte DNA, 2nd ed., Spektrum Akademischer Verlag GmbH, 1993; Watson et al, Recombinant DNA, 2nd ed., W. H. Freeman and Company, 1992; the contents of which are incorporated herein by reference). Monoclonal antibodies useful in the methods of the invention are directed to an epitope of SELADIN-1 or a variant or fragment thereof, such that the complex formed between the antibody and SELADIN-1, or between the antibody and said variant or fragment, can be recognized in detection assays. The term "antibodies" encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, single chain antibodies as well as fragments thereof which specifically bind to SELADIN-1, or to a variant or fragment thereof.

Antibodies or ligands might also be used in detecting specifically molecules mentioned under d) and e) above.

If luminescent labels are used in any detection assay, it is preferred to use a confocal optical set-up.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to e) in a sample from a subject not suffering of said neurological disease. The healthy subject can be of the same weight, age, and gender as the subject who is being diagnosed or prognosed for said neurological disease. In some cases, it might be pref rred to use a ref rence value from th subject which is diagnosed.

In another aspect, the invention features a method of monitoring the progression of a neurological disease in a subject, comprising:
determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of
a) a protein molecule shown in SEQ ID NO. 1,
b) a variant or fragment thereof,
c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, a variant or fragment thereof,
d) a molecule affecting directly or indirectly a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to c),
e) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c),
in a sample from said subject;
and
comparing said level, or said activity, or both said level and said activity, of at least one of said substances a) to e) to a reference value representing a known disease or health status,
thereby monitoring the progression of said neurological disease in said subject.

In a preferred embodiment, the level, or the activity, or both said level and said activity, of at least one of said substances a) to e) in a sample is determined at least twice, e.g. at two points which are weeks or months apart. The levels or activities at these two time points are compared in order to monitor the progression of said neurological disease. It might be preferred to take a series of samples over a period of time. In further preferred embodiments, said subject receives a treatment prior to one or more of said sample gatherings.

In preferred embodiments, said subjects suffer from Alzheimer's disease and related neurofibrillary disorders, or neurodegenerative states characterized by cell degeneration

or cell death. Further examples of neurological diseases are Parkinson's disease, Huntington disease, Amyotrophic lateralsclerosis, Pick's disease.

It is particularly preferred that said sample is a brain tissue or other body cells including blood cells and skin fibroblasts. According to the present invention, a reduction in the level, or activity, or both said level and said activity, of SELADIN-1 or *SELADIN-1* transcripts in said subject's brain regions affected heavily by neurodegeneration relative to a reference value indicates a diagnosis or prognosis of Alzheimer's disease. Predominantly neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus and the amygdala degenerate in Alzheimer's disease.

It is also particularly preferred that said sample is cerebrospinal fluid or another body fluid.

It might be preferred that said subject has previously been determined to have one or more factors indicating that such subject is afflicted with a neurological disease.

In preferred embodiments, said subject can be a human, an experimental animal, e.g. a rat or a mouse, a domestic animal, or a non-human primate, e.g. a monkey. The experimental animal can be an animal model for a disorder, e.g. a transgenic mouse with an Alzheimer's-type neuropathology.

In preferred embodiments, at least one of said substances is detected using an immunoassay, an enzyme activity assay and/or a binding assay.

In preferred embodiments, measurement of the level of transcription products of the *SELADIN-1* gene, or a variant thereof, is performed in body cells using Northern blots with probes specific for the *SELADIN-1* gene or said variant. Quantitative PCR with primer combinations to amplify *SELADIN-1* gene-specific sequences from cDNA obtained by reverse transcription of RNA extracted from body cells of a subject can also be applied. These techniques are known to those of ordinary skill in the art (see e.g. Watson et al., Rekombinant DNA, 2nd edition, Spektrum Akademischer Verlag GmbH,

Heidelberg, 1993; Watson et al., Recombinant DNA, 2nd ed. W.H. Freeman and Company, 1992).

In preferred embodiments, said level or activity of the protein molecule shown in SEQ ID NO. 1, or a variant or fragment thereof, is detected using an immunoassay. These assays can measure the amount of binding between said protein molecule ("SELADIN-1") and an anti-SELADIN-1 antibody by the use of enzymatic, chromodynamic, radioactive, or luminescent labels which are attached to either the anti-SELADIN-1 antibody or a secondary antibody which binds the anti-SELADIN-1 antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow et al., Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

The antibody or ligand to be used should preferably specifically detect SELADIN-1 or a variant or fragment thereof. It is preferred that it does not substantially interact with any other protein present in said sample.

Monoclonal antibodies capable of recognizing a protein molecule of SEQ ID NO. 1 or a variant or fragment thereof can be prepared using methods known in the art (see e.g. Köhler and Milstein, Nature 256, 495 - 497 1975; Kozbor et al., Immunol. Today 4, 72, 1983; Cole et al., Monoclonal antibodies and cancer therapy, Alan R. Liss, Inc., pp 77 - 96, 1985; Marks et al., J. Biol. Chem., 16007 - 16010, 1992; the contents of which are incorporated herein by reference). Such monoclonal antibodies or fragments thereof can also be produced by alternative methods known to those of skill in the art of recombinant DNA technology (see e.g. Sastry et al, PNAS 86: 5728, 1989; ; Watson et al., Rekombinierte DNA, 2nd ed., Spektrum Akademischer Verlag GmbH, 1993; Watson et al, Recombinant DNA, 2nd ed., W. H. Freeman and Company, 1992; the contents of which are incorporated herein by reference). Monoclonal antibodies useful in the methods of the invention ar directed to an epitope of SELADIN-1 or a variant or fragm nt thereof, such that the complex formed betwe n the antibody and SELADIN-1, or betw en the antibody and said variant or fragment, can be r cogniz d in d tection

assays. The term "antibodies" encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, single chain antibodies as well as fragments thereof which specifically bind to SELADIN-1, or to a variant or fragment thereof.

Antibodies or ligands might also be used in detecting specifically any molecules mentioned under d) to e) above.

If luminescent labels are used in any detection assay, it is preferred to use a confocal optical set-up.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to e) in a sample from a subject not suffering of said neurological disease. The healthy subject can be of the same weight, age, and gender as the subject who is being diagnosed or prognosed for said neurological disease. In some cases, it might be preferred to use a reference value from the subject which is diagnosed.

In still another aspect, the invention features a method of evaluating a treatment for a neurological disease, comprising:
determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of
a) a protein molecule shown in SEQ ID NO. 1,
b) a variant or fragment thereof,
c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, a variant or fragment thereof,
d) a molecule affecting directly or indirectly a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to c),
e) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c),
in a sample from said subject;

and

comparing said lev l, or said activity, or both said level and said activity, of at least one of said substances a) to e) to a reference value representing a known disease or health status,

thereby evaluating said treatment for said neurological disease.

In preferred embodiments, said subjects suffer from Alzheimer's disease and related neurofibrillary disorders, or neurodegenerative states characterized by cell degeneration or cell death. Further examples of neurological diseases are Parkinson's disease, Huntington disease, Amyotrophic lateralsclerosis, Pick's disease.

It is particularly preferred that said sample is a brain tissue or other body cells including blood cells and skin fibroblasts. According to the present invention, a reduction in the level, or activity, or both said level and said activity, of SELADIN-1 or *SELADIN-1* transcripts in said subject's brain regions affected heavily by neurodegeneration relative to a reference value indicates a diagnosis or prognosis of Alzheimer's disease. Predominantly neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus and the amygdala degenerate in Alzheimer's disease.

It is also particularly preferred that said sample is cerebrospinal fluid or another body fluid.

It might be preferred that said subject has previously been determined to have one or more factors indicating that such subject is afflicted with a neurological disease.

In preferred embodiments, said subject can be a human, an experimental animal, e.g. a rat or a mouse, a domestic animal, or a non-human primate, e.g. a monkey. The experimental animal can be an animal model for a disorder, e.g. a transgenic mouse with an Alzheimer's-type neuropathology.

In preferred embodiments, at least on of said substances is detected using an immunoassay, an nzyme activity assay and/ r a binding assay.

In preferred embodiments, measurement of the level of transcription products of the *SELADIN-1* gene, or a variant thereof, is performed in body cells using Northern blots with probes specific for the *SELADIN-1* gene or said variant. Quantitative PCR with primer combinations to amplify *SELADIN-1* gene-specific sequences from cDNA obtained by reverse transcription of RNA extracted from body cells of a subject can also be applied. These techniques are known to those of ordinary skill in the art (see e.g. Watson et al., Rekombinierte DNA, 2nd edition, Spektrum Akademischer Verlag GmbH, Heidelberg, 1993; Watson et al., Recombinant DNA, 2nd ed. W.H. Freeman and Company, 1992).

In preferred embodiments, said level or activity of the protein molecule shown in SEQ ID NO. 1, or a variant or fragment thereof, is detected using an immunoassay. These assays can measure the amount of binding between said protein molecule ("SELADIN-1") and an anti-SELADIN-1 antibody by the use of enzymatic, chromodynamic, radioactive, or luminescent labels which are attached to either the anti-SELADIN-1 antibody or a secondary antibody which binds the anti-SELADIN-1 antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow et al., Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

The antibody or ligand to be used should preferably specifically detect SELADIN-1 or a variant or fragment thereof. It is preferred that it does not substantially interact with any other protein present in said sample.

Monoclonal antibodies capable of recognizing a protein molecule of SEQ ID NO. 1 or a variant or fragment thereof can be prepared using methods known in the art (see e.g. Köhler and Milstein, Nature 256, 495 - 497 1975; Kozbor et al., Immunol. Today 4, 72, 1983; Cole et al., Monoclonal antibodies and cancer therapy, Alan R. Liss, Inc., pp 77 - 96, 1985; Marks et al., J. Biol. Chem., 16007 - 16010, 1992; the contents of which are incorporated herein by reference). Such monoclonal antibodies or fragments thereof can

also be produced by alternative methods known to those of skill in the art of recombinant DNA technology (see e.g. Sastry et al, PNAS 86: 5728, 1989; ; Watson et al., Rekombinierte DNA, 2nd ed., Spektrum Akademischer Verlag GmbH, 1993; Watson et al, Recombinant DNA, 2nd ed., W. H. Freeman and Company, 1992; the contents of which are incorporated herein by reference). Monoclonal antibodies useful in the methods of the invention are directed to an epitope of SELADIN-1 or a variant or fragment thereof, such that the complex formed between the antibody and SELADIN-1, or between the antibody and said variant or fragment, can be recognized in detection assays. The term "antibodies" encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, single chain antibodies as well as fragments thereof which specifically bind to SELADIN-1, or to a variant or fragment thereof.

Antibodies or ligands might also be used in detecting specifically any molecules mentioned under d) to e) above.

If luminescent labels are used in any detection assay, it is preferred to use a confocal optical set-up.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to e) in a sample from a subject not suffering of said neurological disease. The healthy subject can be of the same weight, age, and gender as the subject who is being diagnosed or prognosed for said neurological disease. In some cases, it might be preferred to use a reference value from the subject which is diagnosed.

In a further aspect, the invention features a kit for diagnosis, or prognosis, of a neurological disease, said kit comprising:
(1) at least one reagent which is selected from the group consisting of reagents that selectively detect
a) a protein molecule shown in SEQ ID NO.1,
b) a variant or fragment ther of,

c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO.1, a variant or fragment thereof,

d) a molecule affecting directly or indirectly a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) t c),

) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c), and

(2) instructions for diagnosing, or prognosing said neurological disease by

(i) detecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to e) in a sample from said subject;

and

(ii) diagnosing, or prognosing said neurological disease, wherein a varied level, or varied activity, or both said varied level and said varied activity, of at least one substance which is selected from the group consisting of a) to e) compared to a reference value representing a known health status; or a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to e) similar or equal to a reference value representing a known disease status indicates diagnosis, or prognosis of said neurological disease.

In preferred embodiments, said subjects suffer from Alzheimer's disease and related neurofibrillary disorders, or neurodegenerative states characterized by cell degeneration or cell death. Further examples of neurological diseases are Parkinson´s disease, Huntington disease, Amyotrophic lateralsclerosis, Pick´s disease.

It is particularly preferred that said sample is a brain tissue or other body cells including blood cells and skin fibroblasts. According to the present invention, a reduction in the lev l, or activity, or both said level and said activity, of *SELADIN-1* transcripts in said subject's brain regions affected heavily by neurodegeneration relative to a reference value indicates a diagnosis or prognosis of Alzheimer's dis as . Predominantly neurons

within the inf rior t mporal lobe, the entorhinal cortex, the hippocampus and the amygdala degenerate in Alzheimer's disease.

It is also particularly preferred that said sample is cerebrospinal fluid or another body fluid.

It might be preferred that said subject has previously been determined to have one or more factors indicating that such subject is afflicted with a neurological disease.

In preferred embodiments, said subject can be a human, an experimental animal, e.g. a rat or a mouse, a domestic animal, or a non-human primate, e.g. a monkey. The experimental animal can be an animal model for a disorder, e.g. a transgenic mouse with an Alzheimer's-type neuropathology.

In preferred embodiments, at least one of said substances a) to e) is detected using an immunoassay, an enzyme activity assay and/or a binding assay.

In preferred embodiments, measurement of the level of transcription products of the *SELADIN-1* gene, or a variant thereof, is performed in body cells using Northern blots with probes specific for the *SELADIN-1* gene or said variant. Quantitative PCR with primer combinations to amplify *SELADIN-1* gene-specific sequences from cDNA obtained by reverse transcription of RNA extracted from body cells of a subject can also be applied. These techniques are known to those of ordinary skill in the art (see e.g. Watson et al., Rekombinierte DNA, 2nd edition, Spektrum Akademischer Verlag GmbH, Heidelberg, 1993; Watson et al., Recombinant DNA, 2nd ed. W.H. Freeman and Company, 1992).

In preferred embodiments, said level or activity of the protein molecule shown in SEQ ID NO. 1, or a variant or fragment th reof, is detected using an immunoassay. These assays can m asure the amount of binding between said protein molecule ("SELADIN-1") and an anti-SELADIN-1 antibody by the use of enzymatic, chromodynamic, radioactive, or luminescent labels which ar attached to eith r the anti-SELADIN-1

antibody or a secondary antibody which binds the anti-SELADIN-1 antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow et al., Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

The antibody or ligand to be used should preferably specifically detect SELADIN-1 or a variant or fragment thereof. It is preferred that it does not substantially interact with any other protein present in said sample.

Monoclonal antibodies capable of recognizing a protein molecule of SEQ ID NO. 1 or a variant or fragment thereof can be prepared using methods known in the art (see e.g. Köhler and Milstein, Nature 256, 495 - 497 1975; Kozbor et al., Immunol. Today 4, 72, 1983; Cole et al., Monoclonal antibodies and cancer therapy, Alan R. Liss, Inc., pp 77 - 96, 1985; Marks et al., J. Biol. Chem., 16007 - 16010, 1992; the contents of which are incorporated herein by reference). Such monoclonal antibodies or fragments thereof can also be produced by alternative methods known to those of skill in the art of recombinant DNA technology (see e.g. Sastry et al, PNAS 86: 5728, 1989; ; Watson et al., Rekombinierte DNA, 2nd ed., Spektrum Akademischer Verlag GmbH, 1993; Watson et al, Recombinant DNA, 2nd ed., W. H. Freeman and Company, 1992; the contents of which are incorporated herein by reference). Monoclonal antibodies useful in the methods of the invention are directed to an epitope of SELADIN-1 or a variant or fragment thereof, such that the complex formed between the antibody and SELADIN-1, or between the antibody and said variant or fragment, can be recognized in detection assays. The term "antibodies" encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, single chain antibodies as well as fragments thereof which specifically bind to SELADIN-1, or to a variant or fragment thereof.

Antibodies or ligands might also be used in detecting specifically any molecules mentioned under d) to e) above.

If luminescent labels are used in any d tection assay, it is preferred to use a confocal optical set-up.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to e) in a sample from a subject not suffering of said neurological disease. The healthy subject can be of the same weight, age, and gender as the subject who is being diagnosed or prognosed for said neurological disease. In some cases, it might be preferred to use a reference value from the subject which is diagnosed.

In another aspect, the kit may be used in monitoring a progression of said neurological disease in a subject.

In a further aspect, the kit may be used in monitoring success or failure of a therapeutic treatment of said subject.

In another aspect, the invention features a method of treating or preventing a neurological disease in a subject comprising administering to said subject in a therapeutically effective amount an agent or agents which directly or indirectly affect a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of

a) a protein molecule shown in SEQ ID NO.1,

b) a variant or fragment thereof,

c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO.1, a variant or fragment thereof,

d) a molecule affecting directly or indirectly a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to c),

) a molecule which is affected in its level, or its activity, or both its lev l and activity, by at least one substance which is selected from the group consisting of a) to c).

In preferred embodiments, the method comprises the application of per se known methods of gene therapy and/or antisense nucleic acid technology to administer said agent or said agents.

In general, gene therapy includes several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein, and modulation of endogeneous cellular gene expression by recombinant expression methods or by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, Acc. Chem. Res. 26, 274 - 278, 1993; Mulligan, Science 260, 926 - 931, 1993; the contents of which are incorporated herein by reference) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane perturbation by chemical (solvents, detergents, polymers, enzymes) or physical means (mechanic, osmotic, thermic, electric shocks). The postnatal gene transfer into the central nervous system has been described in detail (see e.g. Wolff, Current Opinion in Neurobiology, 3, 743 - 748, 1993; the contents of which are incorporated herein by reference).

In particular, the invention features a method of treating or preventing a neurological disease, such as Alzheimer's disease, by means of antisense nucleic acid therapy, i.e. the down-regulation of an inappropriately expressed or defective gene by the introduction of antisense nucleic acids or derivatives thereof into certain critical cells (see e.g. Gillespie, DN & P 5(7), 389 - 395, 1992; Agrawal, Tibtech 13, 197 - 199, 1995; Crooke, Bio/Technology 10, 882 - 886, 1992; the contents of which are incorporated herein by reference). Apart from hybridization strategies, the application of ribozymes, i.e. RNA molecules that act as enzymes, destroying RNA that carries the message of disease has also been described (see e.g. Barinaga, Science, 262, 1512 - 1514, 1993; the contents of which are incorporated herein by reference). It is particularly preferred that cells of the central nervous system, preferably the brain, of a subject are treated in such a way. Cell penetration can be performed by known strategies such as coupling of antisense nucleic acids and derivatives thereof to carrier particl s, or the above

described techniques. Strategies for administering targeted therapeutic oligodeoxynucleotides are known to those of skill in the art (see e.g. Wickstrom, Tibtech, 10, 281 - 287, 1992; the contents of which are incorporated herein by reference). In some cases, delivery can be performed by mere topical application. Further approaches are directed to intracellular expression of antisense RNA. In this strategy, cells are transformed *ex vivo* with a recombinant gene that directs the synthesis of an RNA that is complementary to a region of the target nucleic acid. Therapeutically use of intracellularly expressed antisense RNA is procedurally similar to gene therapy.

In preferred embodiments, the method comprises grafting donor cells into the central nervous system, preferably the brain, of said subject, said subject or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent or agents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be performed by electroporation, chemically mediated transfection, in particular calcium phospate transfection, liposomal mediated transfection, etc.

In preferred embodiments, said agent is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising introducing subject cells into said subject, said subject cells having been treated *in vitro* to insert a DNA segment encoding said therapeutic protein, said subject cells expressing *in vivo* in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells *in vitro* by a viral vector, in particular a retroviral vector.

In preferred embodiments, the therapeutic nucleic acid or protein reduces or prevents the degeneration of neurons and slowes brain amyloid formation.

In another aspect, the invention features an agent which directly or indirectly affects an activity, or level, or both said activity or level, of at least one substance which is selected from the group consisting of

a) a protein molecule shown in SEQ ID NO.1,

b) a variant or fragment thereof,

c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO.1, a variant or fragment thereof,

d) a molecule affecting directly or indirectly a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to c),

e) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c).

In another aspect, the invention features a medicament comprising such an agent.

In still another aspect, the invention features an agent for treating or preventing a neurological disease, which agent directly or indirectly affects an activity, or level, or both said activity or level, of at least one substance which is selected from the group consisting of

a) a protein molecule shown in SEQ ID NO.1,

b) a variant or fragment thereof,

c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO.1, a variant or fragment thereof,

d) a molecule affecting directly or indirectly a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to c),

e) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c).

In preferred embodiments, said neurological diseases are Alzheimer's disease and related neurofibrillary disorders, or neurodegenerative states characterized by cell

degeneration or cell death. Further xamples of neurological diseases are Parkinson's disease, Huntington disease, Amyotrophic lateralsclerosis, Pick's disease.

In a further aspect, the invention features the use of an agent , for treating or preventing a neurological disease, which agent directly or indirectly affects an activity, or level, or both said activity or level, of at least one substance which is selected from the group consisting of

a) a protein molecule shown in SEQ ID NO.1,

b) a variant or fragment thereof,

c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO.1, a variant or fragment thereof,

d) a molecule affecting directly or indirectly a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to c),

e) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c).

In preferred embodiments, said neurological diseases are Alzheimer's disease and related neurofibrillary disorders, or neurodegenerative states characterized by cell degeneration or cell death. Further examples of neurological diseases are Parkinson's disease, Huntington disease, Amyotrophic lateralsclerosis, Pick's disease.

In a further aspect, the invention features a method for identifying an agent that directly or indirectly affects an activity, or level, or both said activity or level, of at least one substance which is selected from the group consisting of

a) a protein molecule shown in SEQ ID NO.1,

b) a variant or fragment thereof,

c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO.1, a variant or fragment thereof,

d) a molecule affecting directly or indirectly a level, or an activity, or both said level and said activity, of at l ast one substance which is selected from th group consisting of a) to c).

e) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c),

comprising the steps of:

(i) providing a sample containing at least one substance which is selected from the group consisting of a) to e);

(ii) contacting said sample with at least one agent;

(iii) comparing an activity, or level, or both said activity and level, of at least one of said substances before and after said contacting.

Figure 1 depicts the selective vulnerability of brain regions in Alzheimer's disease. Predominantly neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus and the amygdala degenerate in Alzheimer's disease (Terry et al., Annals of Neurology, 10, 184-192, 1981). These brain regions are predominantly involved in the processing of learning and memory functions. In contrast, neurons within the frontal cortex, the occipital cortex and the cerebellum are largely intact and preserved from the n urodegenerative process in Alzheimer's disease.

Figure 2 discloses the identification of genes differentially expressed in brain regions from Alzheimer's disease patients. Brain areas with massive neuronal cell loss as well as areas with largely preserved neurons were identified and RNA extracted. Synthesis of cDNA was performed using an oligo-dT primer followed by PCR using the oligo-dT primer in combination with random primers and (α^{35}S)-dATP. Reactions were separated on DNA sequencing gels, DNA bands visualized by autoradiography and bands lighting up in different intensities were cut out. DNA fragments were reamplified by PCR, cloned in *E. coli* and sequences determined. Expression and functional analyses were performed.

Figure 3 depicts the specifications of Alzheimer's disease brain tissue as it was used in the examples. Brain tissues from Alzheimer's disease patients and control subjects were removed within 6 hours of death, and immediately frozen on dry ice. For RNA extraction tissue sections from the inferior temporal lobe and frontal cortex were used.

Figure 4 discloses the quantification of *SELADIN-1* transcripts in brain tissue from Alzheimer's disease and control subjects by Northern blot analyses. Transcript levels were significantly lower in brain regions with severe neurodegeneration, i. e. temporal lobe in Alzheimer's disease (AD1-3) but not in normal brain (NB1-3), as compared to protected brain regions, i. e. frontal lobe. This decrease was specific as indicated by unchanged β-actin transcript levels used to control for equal loading of RNA.

Figure 5 depicts the transcription levels of the *SELADIN-1* gene in different human brain regions. The *SELADIN-1* gene was found to be expressed throughout the human brain. In particular transcription levels are high in all cortical areas, the hippocampus, the amygdala, the spinal cord, and the medulla. Note the unchanged levels in temporal lobe versus frontal lobe in this brain derived from a cognitively normal control subject without any signs of Alzheimer's disease. The analysis of β-actin transcripts was used as loading control.

Figure 6 depicts the distribution of *SELADIN-1* transcripts in human tissues. Comparable samples of RNA were spotted on nitrocellulose filters and *SELADIN-1* transcripts were quantified by hybridization using a labeled SELADIN-1 gene specific probe. Significant levels of *SELADIN-1* gene transcripts were found in all brain regions tested. Transcripts were also detected in other tissues, however, strong variations in signal intensity indicated a tissue specific regulation of *SELADIN-1* expression.

Figure 7 depicts the expression of the *SELADIN-1* gene in rat brain cortex, hippocampus and basal nucleus analyzed by in situ hybridization. This staining pattern along with the higher magnifications indicate that *SELADIN-1* is predominantly expressed in neurons. No significant hybridization signals were observed with glial cells.

Figure 8 depicts the expression of *SELADIN-1* in rat brain nuclei. Strong *SELADIN-1* expression was found in the occulomotor, paraventricular, red and facial nuclei. Higher magnifications indicate predominant hybridization with neurons. No significant hybridization signals wer observed with glial cells.

Figure 9 depicts the expression of *SELADIN-1* in rat brain hippocampus and substantia nigra. In situ hybridization with *SELADIN-1* transcripts was detected by photoemulsion-autoradiography, confirming the neuron specific expression of this gene.

Figure 10 discloses the subcellular localization of a SELADIN-1-EGFP (enhanced green fluorescent protein) fusion in transfected Cos cells. The confocal micrographs show the co-localization of the SELADIN-1-EGFP fusion with the golgi specific stain BODIPY TR ceramide indicating localization of SELADIN-1 in the Golgi apparatus and the endoplasmic reticulum.

Figure 11 discloses that the SELADIN-1-EGFP fusion does not localize to mitochondria in transfected cos cells in spite of a putative mitochondrial targeting sequence close to the N-terminus of the SELADIN-1 protein. The confocal micrographs show the different staining patterns caused by the SELADIN-1-EGFP fusion and the specific mitochondrial stain Mito Tracker Red CM-H_2XRos.

Figure 12 discloses structural features of the SELADIN-1 protein based on multiple sequence alignments and secondary structure predictions. Near the N-terminus the SELADIN-1 protein contains a putative mitochondrial localization signal that appears to be inactive in transfected Cos cells or when used in EGFP fusions. The central region of the protein contains a sequence that is homologous to a family of oxidoreductases and that contains a FAD site for covalent binding. The protein is predicted to contain five transmembrane regions. The expression in neurons, the co-localization in the Golgi apparatus and the endoplasmic reticulum of the SELADIN-1 protein, the amyloid precursor protein (APP) and the presenilins PS1 and PS2 and furthermore the transmembrane character suggest a functional relationship between these proteins. Mutations in both APP and presenilins were shown to cause an increase in the production of β-amyloid. In a similar way the SELADIN-1 protein might be involved in common biological pathways influencing the processing of the amyloid precursor protein and the generation of Aβ. Using the SELADIN-1 protein as a probe, interaction partners can be identified which might represent new AD drug targets.

Figure 13 discloses the protein sequence of SELADIN-1 (SEQ ID NO. 1). The full length protein consists of 516 amino acid residues. The sequence is given in the one letter amino acid code.

Figure 14 discloses the nucleotide sequence of the cloned *SELADIN-1* cDNA (SEQ ID NO. 2) comprising 4248 nucleotides. The coding sequence for the SELADIN-1 protein starts at nucleotide position 100 and stops at position 1648.

Figure 15 discloses the comparison of nucleotide sequences of the cloned *SELADIN-1* cDNA comprising 4248 nucleotides and the *KIAA0018* cDNA comprising 4186 nucleotides. A significant difference exists at position 1228 of the *SELADIN-1* sequence where a C nucleotide (C/G basepair) is missing in the *KIAA0018* sequence. This results in a frameshift in the open reading frame in the *KIAA0018* sequence relative to the *SELADIN-1* sequence. The consequence is that the translation product of the *KIAA0018* gene is 390 amino acids in length compared to 516 amino acid residues of the SELADIN-1 translation product. In addition to the difference in length, the frameshift causes a difference between the C-terminal 14 amino acids of the KIAA0018 protein and the corresponding sequence area of the SELADIN-1 polypeptide (pos. 377 - 390). The coding sequence for the SELADIN-1 protein starts at nucleotide position 100 and stops at position 1648.

EXAMPLE I

Post-mortem Alzheimer's disease brain tissues

Brain tissues from Alzheimer's disease patients and control subjects were removed within 6 hours of death, and immediately frozen on dry ice. Parallel sections were fixed in formaldehyde for histopathological confirmation of the diagnosis and for cell counts. Brain areas with massive neuronal cell loss as well as areas with largely preserved neurons were identified for comparisons of gene expression and stored at -80°C until RNA extractions were performed.

Identification of *SELADIN-1* by differential display PCR

Total RNA from post-mortem brain tissues was prepared by using the RNeasy kit (Qiagen). The RNA preparations were treated with DNase I (Boehringer Mannheim) together with RNAsin (Promega) for 30 minutes, followed by phenol extraction, and ethanol precipitation. 0.2 mg of each RNA preparation were transcribed to cDNA by using Expand Reverse Transcriptase (Boehringer Mannheim) with one base ancor primers $HT_{11}A$, $HT_{11}C$ and $HT_{11}G$. In the following PCR reaction, the cDNAs were amplified by using $HT_{11}A$ along with the random primers HAP-5 (5'-TGCCGAAGCTTGGAGCTT-3') and HAP3-T (5'TGCCGAAGCTTTGGTCAT-3'). Taq-polymerase (AmpliTaq, Perkin Elmer Corp.), dGTP, dCTP, and dTTP (Amersham Pharmacia Biotech) and (α^{35}S)-dATP (NEN life science products) were used in a PCR protocol according to Zhao et al. The PCR products were separated on 6% polyacrylamide-urea sequencing gels that were dried subsequently on 3 mm filter paper (Whatman), and X-ray films (Dupont) were exposed for 12 hours.

Cloning and sequencing

Differential bands were excised from the gel, boiled in water for 10 minutes, centrifuged, and cDNAs were precipitated from the supernatant fluids by using ethanol and glycogen/sodiumacetate, followed by dialysis against 10% glycerol for 1 hour through 0.025 mm filters (type VS, Millipore). The dialysates were used as templates for the reamplification reactions that were done under identical conditions as in the differential

display PCR, with the exception of th initial cycle for nonspecific annealing. The resulting PCR products were separated by agarose gelelectrophoresis, purified from the gel with the QIAEXII Agarose Gel Extraction Kit (Qiagen), and cloned into the *Hind* III restriction site of pBluescript KS (Stratagene). Cloned cDNA fragments were sequenced with an ABI 377 DNA sequencer (Perkin Elmer Corp.) by using T3 and T7 primers.

Amplification of a *SELADIN-1* cDNA-fragment

A *SELADIN-1* cDNA fragment was amplified by using cDNA transcribed from human brain tissue by using RNA High Fidelity Taq-polymerase (Boehringer Mannheim) and *SELADIN-1*-specific primers for a PCR reaction with 40 cycles of annealing at 70 °C for 1 minute, and polymerization at 72 °C for 3 minutes. The PCR products were separated by agarose gel electrophoresis, purified, and cloned into the *Sma* I restriction site of pBluescript KS (Stratagene). The cloned *SELADIN-1* PCR product was sequenced, and restriction fragments were used as probes both for screening a human brain cDNA library and for probing Northern blots.

Northern blotting

Total RNA from post-mortem human brains were prepared by using the Trizol reagent (Gibco BRL, Life Technologies), following the manufacturer's instructions. 5 - 10 mg of RNA were separated in 1 % formaldehyde-containing agarose gels, and the RNA was blotted onto nylon membranes (Hybond-N^+, Amersham). Membranes were hybridized with (α^{32}P)-dCTP (NEN) labeled *SELADIN-1*-specific cDNA probes that were generated by using the Megaprime DNA labelling kit (Amersham). Membranes were washed under high stringency conditions, and X-ray films were exposed for 1 to 72 hours. To control for equal loading of RNA, the identical membranes were probed with a 700 bp cDNA fragment of human glycerolaldehyd-3-phosphate dehydrogenase (*GAPDH*), or with a β-actin cDNA fragment (Clontech).

In situ hybridization

Several *SELADIN-1*-specific cDNA probes of 650bp and of 900bp representing the initial two parts of the open reading frame were cloned in pBluescript (Stratagene) and reversely transcribed in the presence of (α^{35}S)-CTP by using the Ambion transcription kit. In situ hybridization was done with, 14mm sections of adult rat brain cut on a cryomicrotome, mounted on aminoalkylsilane-treated slides and fixed in 4 % paraformaldehyde in PBS for 5 min at room temperature. After washing for 5 min in PBS, sections were acetylated for 10 min, passed through a series of increasing ethanol grades and air dried. Prehybridizations were done in 50 % deionized formamide, 25 mM EDTA, 25 mM Pipes, pH 6.8, 0.75 M NaCl, 0.2 % SDS, 5xDenhardt's, 10 mM DTT, 250 mg/ml denatured herring sperm DNA and 250 mg/ml yeast tRNA. Hybridization of slides with RNA sense and antisense probes diluted to 2000 – 5000 cpm/ml in the same buffer with additional 10 % dextransulphate was performed at 50°C for 12 hours. Slides were then washed four times in 4xSCC for 5 min. each, followed by an incubation for 30 min. at 37 °C with 40 mg/ml RNAseA in 0.5 M NaCl, 10 mM Tris-HCL, pH 7.5, 1mM EDTA and another 30 min without RNAseA. Then slides were washed twice for 15 min. at 50°C in 2xSCC and dried through graded ethanols. Slides were exposed to Kodak Biomax x-ray films for 15 days and subsequently dipped in Kodak NTB-3 nuclear track emulsion and exposed for 6 weeks. After developing in Kodak D19 and fixing in Kodak Unifix, slides were counterstained with Mayer" Hemalaun and coverslipped.

Recombinant expression of SELADIN-1-EGFP fusion proteins in tissue culture

The complete coding region of *SELADIN-1* was subcloned into the N-terminus of the pEGFP-N1-expression vector (Clontech). Cos-7-cells were transfected with *EGFP* or with *SELADIN-1-EGFP* by using the SuperFect transfection reagent from Qiagen according to the manufacturers instructions. Cells were cultured in 3 cm dishes for two days. Part of the cells were stained for the Golgi-apparatus with 0.25 mM BODIPY TR ceramide (molecular probes) for one hour, the other part was treated with 250 nM of the mitochondrial stain Mito Tracker Red CM-H2Xros (Molecular probes) for 45 min. the subcellular localization of the SELADIN-1-EGFP fusion protein was analyzed by confocal laser scanning microscopy using the appropriate filter sets.

CLAIMS

1. An isolated nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1.

2. A DNA molecule having a coding sequence as shown in SEQ ID NO. 2 or a coding sequence substantially the same as the coding sequence of SEQ ID NO. 2.

3. A protein molecule shown in SEQ ID NO. 1.

4. A method of diagnosing or prognosing a neurological disease in a subject comprising:
determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of
a) a protein molecule shown in SEQ ID NO. 1,
b) a variant or fragment thereof,
c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, a variant or fragment thereof,
d) a molecule affecting directly or indirectly a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to c),
e) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c)
in a sample from said subject;
and
comparing said level, or said activity, or both said level and said activity, of at least one of said substances a) to e) to a reference value representing a known disease or health status,
thereby diagnosing or prognosing said neurological disease in said subject.

5. The method according to claim 4, wherein said subjects suffers fr m Alzheimer's disease and related neurofibrillary disorders, or neurodegenerative states characterized by cell degeneration or cell death.

6. A method of evaluating a treatment for a neurological disease, comprising:
determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of
a) a protein molecule shown in SEQ ID NO. 1,
b) a variant or fragment thereof,
c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, a variant or fragment thereof,
d) a molecule affecting directly or indirectly a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a) to c),
e) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c)
in a sample from said subject;
and
comparing said level, or said activity, or both said level and said activity, of at least one of said substances a) to e) to a reference value representing a known disease or health status,
thereby evaluating said treatment for said neurological disease.

7. An agent which directly or indirectly affects an activity, or level, or both said activity or level, of at least one substance which is selected from the group consisting of
a) a protein molecule shown in SEQ ID NO.1,
b) a variant or fragment thereof,
c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO.1, a variant or fragment thereof,

d) a molecule affecting directly or indirectly a level, or an activity, or both said level and activity, of at least one substance which is selected from the group consisting of a) to c).

e) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c).

8. A medicament comprising an agent according to claim 7.

9. An agent for treating or preventing a neurological disease, which agent directly or indirectly affects an activity, or level, or both said activity or level, of at least one substance which is selected from the group consisting of

a) a protein molecule shown in SEQ ID NO.1,

b) a variant or fragment thereof,

c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO.1, a variant or fragment thereof,

d) a molecule affecting directly or indirectly a level, or an activity, or both said level and activity of at least one substance which is selected from the group consisting of a) to c),

e) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c).

10. A medicament for treating or preventing a neurological disease comprising an agent according to claim 9.

11. Use of an agent for treating or preventing a neurological disease, which agent directly or indirectly affects an activity, or level, or both said activity or level, of at least one substance which is selected from the group consisting of

a) a protein molecule shown in SEQ ID NO.1,

b) a variant or fragment thereof,

c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO.1, a variant or fragment thereof,

d) a molecule affecting directly or indirectly a level, or an activity, or b th said level and activity of at least one substance which is selected from the group consisting of a) to c),

e) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c).

12. A method for identifying an agent that directly or or indirectly affects an activity, or level, or both said activity or level, of at least one substance which is selected from the group consisting of

a) a protein molecule shown in SEQ ID NO.1,

b) a variant or fragment thereof,

c) a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO.1, a variant or fragment thereof,

d) a molecule affecting directly or indirectly a level, or an activity, or both said level and activity of at least one substance which is selected from the group consisting of a) to c),

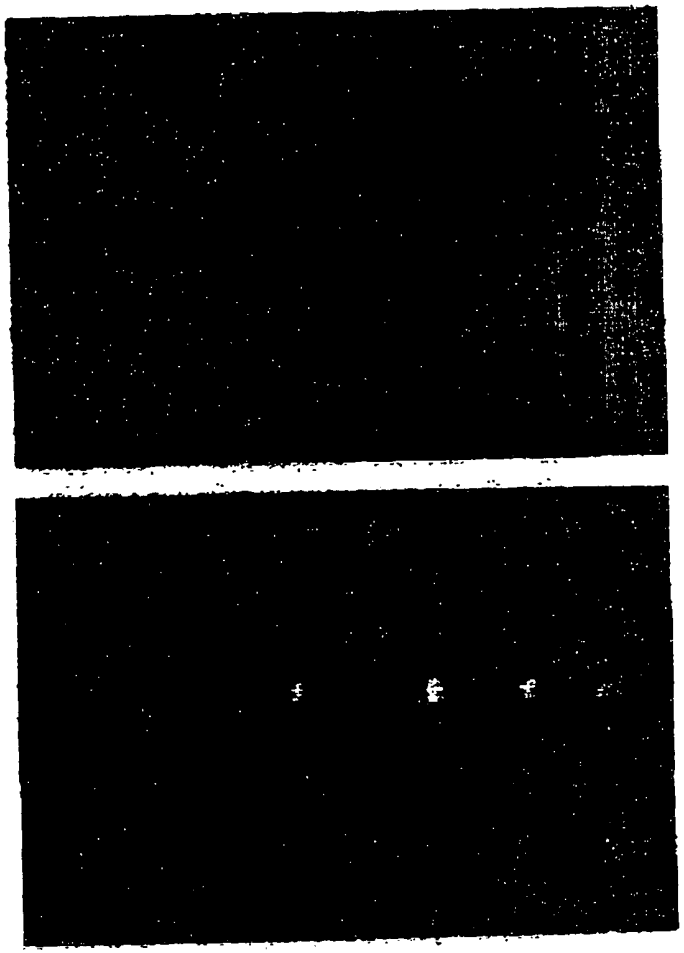
e) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of a) to c) comprising the steps of:

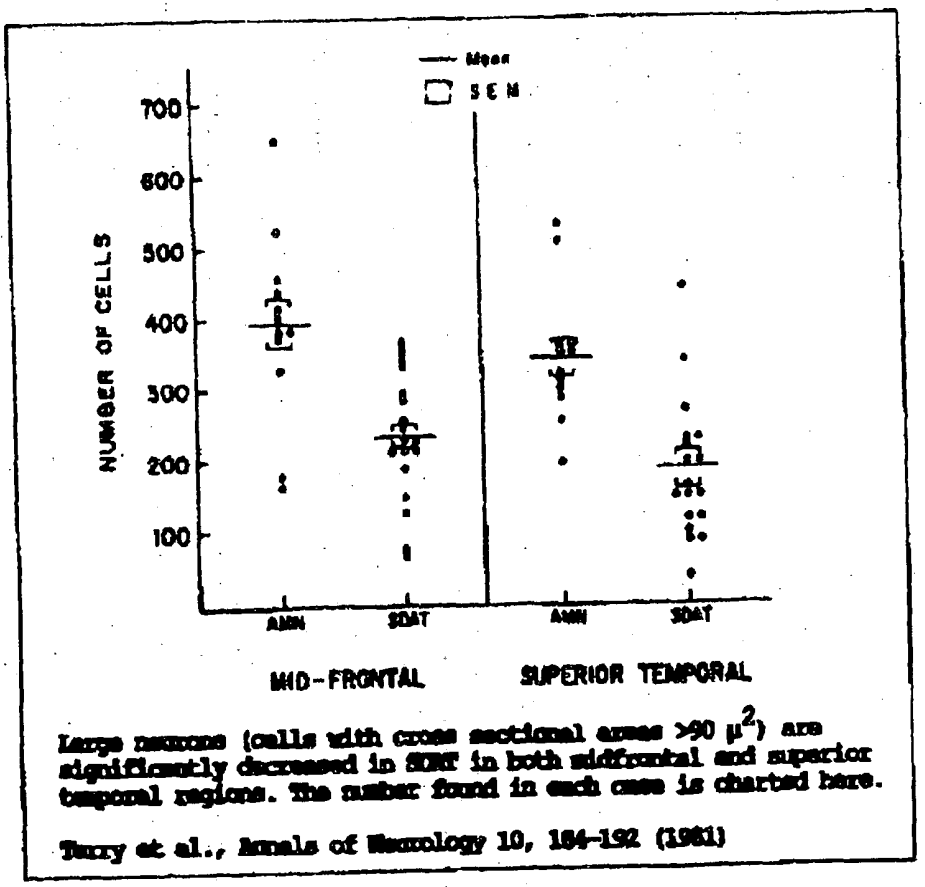
(i) providing a sample containing at least one substance which is selected from the group consisting of a) to e);

(ii) contacting said sample with at least one agent;

(iii) comparing an activity, or level, or both said activity and level, of at least one of said substances before and after said contacting.

Selective vulnerability of brain regions in Alzheimer's disease





Large neurons (cells with cross sectional areas >90 μ^2) are significantly decreased in SDAT in both midfrontal and superior temporal regions. The number found in each case is charted here.

Terry et al., Annals of Neurology 10, 184-192 (1981)

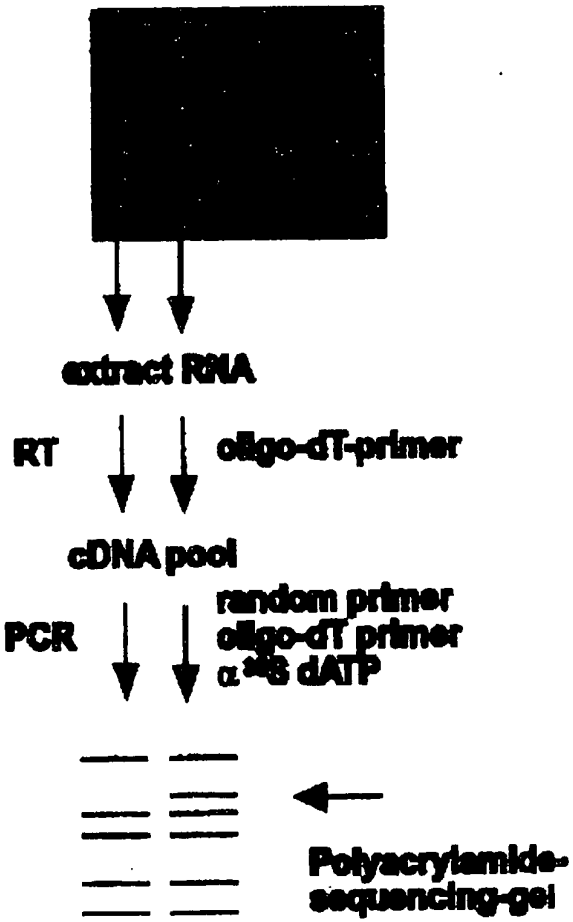
Figure 1

RCV VON:EPA MUENCHEN 06 12-11-1998 15:55 EP9812147 8.6 +49 89 23994465 SPEC

Printed:11-01-2000

12-11-1998 MUENCHEN 06 ; 12-11-98 ; EP9812147B6 221 1342977+ +49 89 239944 SPEC

Identification of gene differentially expressed in AD brain regions



- cut out differentially expressed bands
- reamplify eluted cDNAs
- cloning and sequencing
- expression analysis
- functional analysis

Figure 2

Identification of genes differentially expressed in AD brain regions

Material: AD brain tissue
post mortem time intervall <6h
2 different regions histologically characterized
- inferior temporal lobe
- frontal cortex

Method: mRNA differential display screen

Figur 3

Printed: 1-01-2000

12-11-1998 BENCHEN 06
12-11-98
EP98121478 6 221 134297
+49 89 23994
SPEC

Expression of Seladin-1 in AD brain

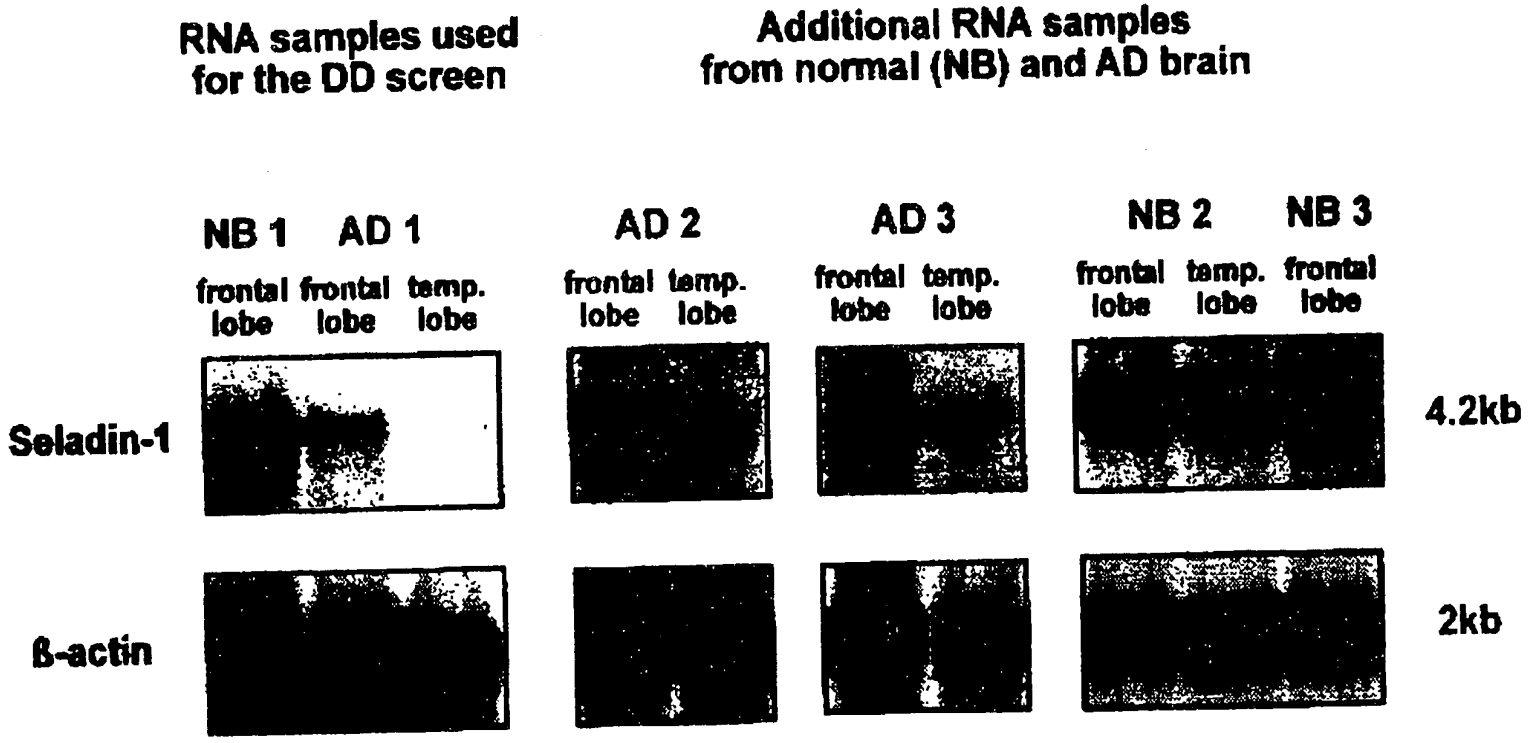
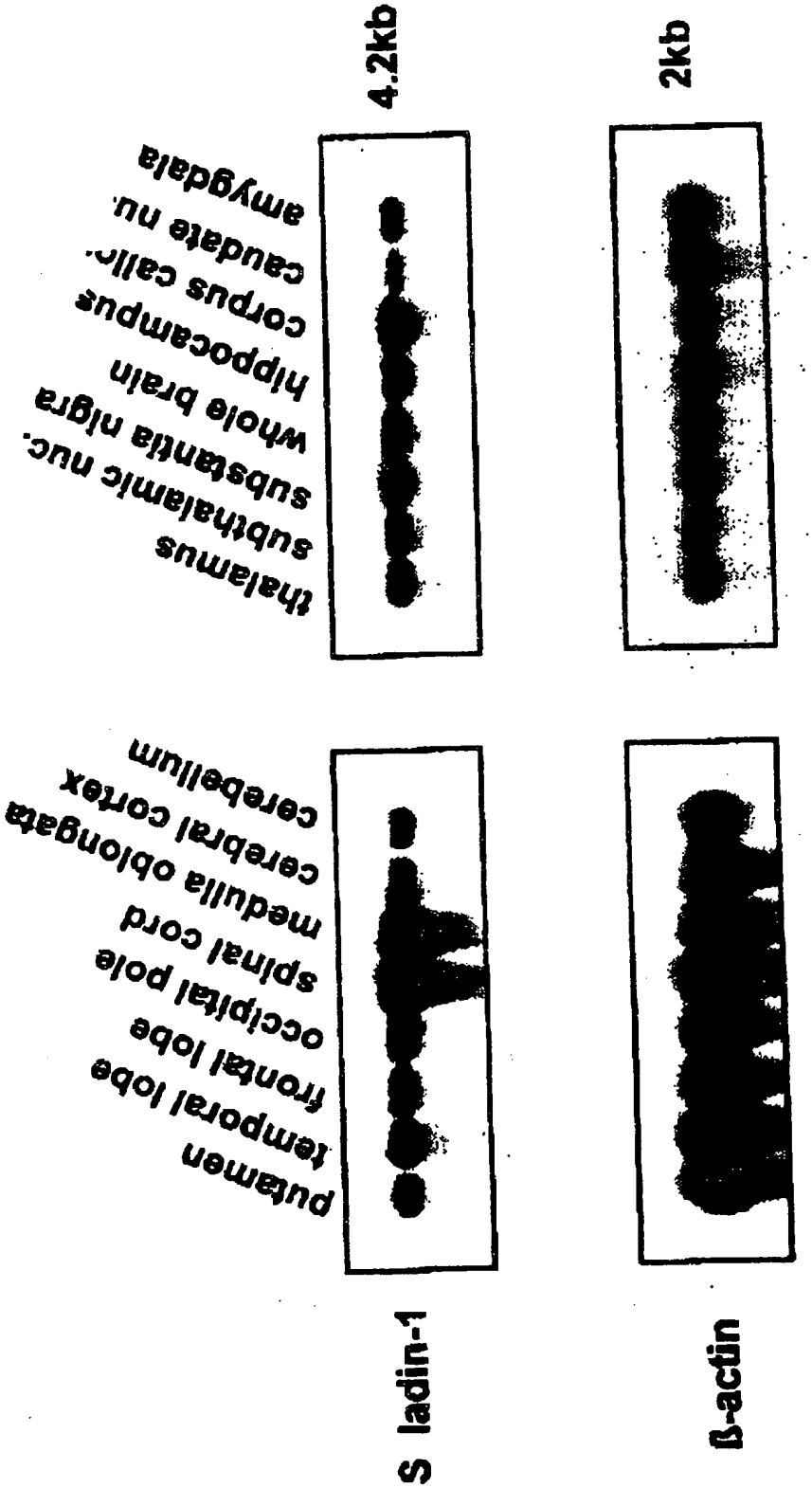
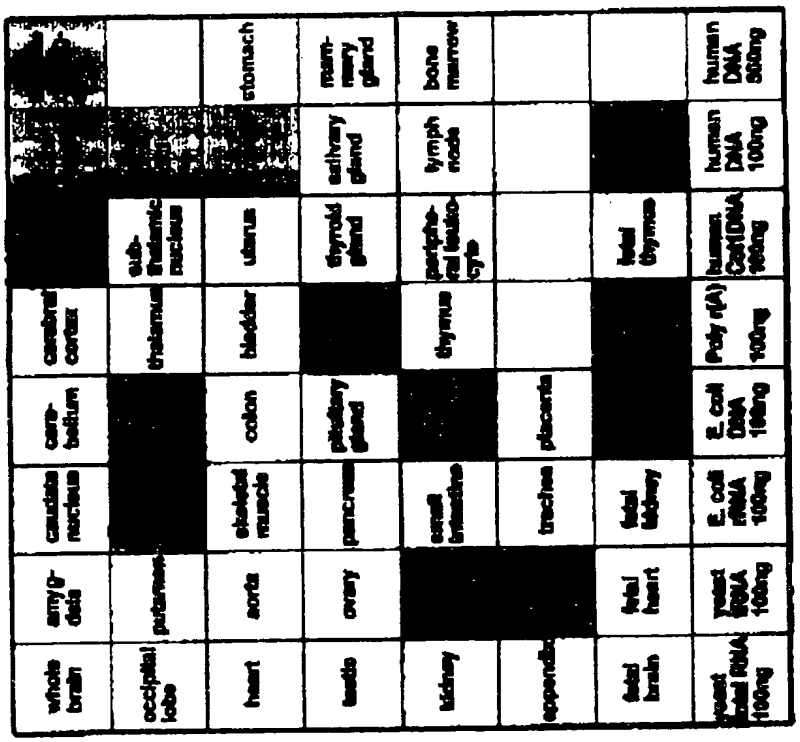


Figure 4

Figure 5


Expression of Seladin-1 in different human brain regions
4.2kb
2kb
thalamus
subthalamic nuc.
substantia nigra
whole brain
hippocampus
corpus calli.
caudate nu.
amygdala
putamen
temporal lobe
frontal lobe
occipital pole
spinal cord
medulla oblongata
cerebral cortex
cerebellum
S ladin-1
β-actin

Expression of Seladin-1 in human tissues



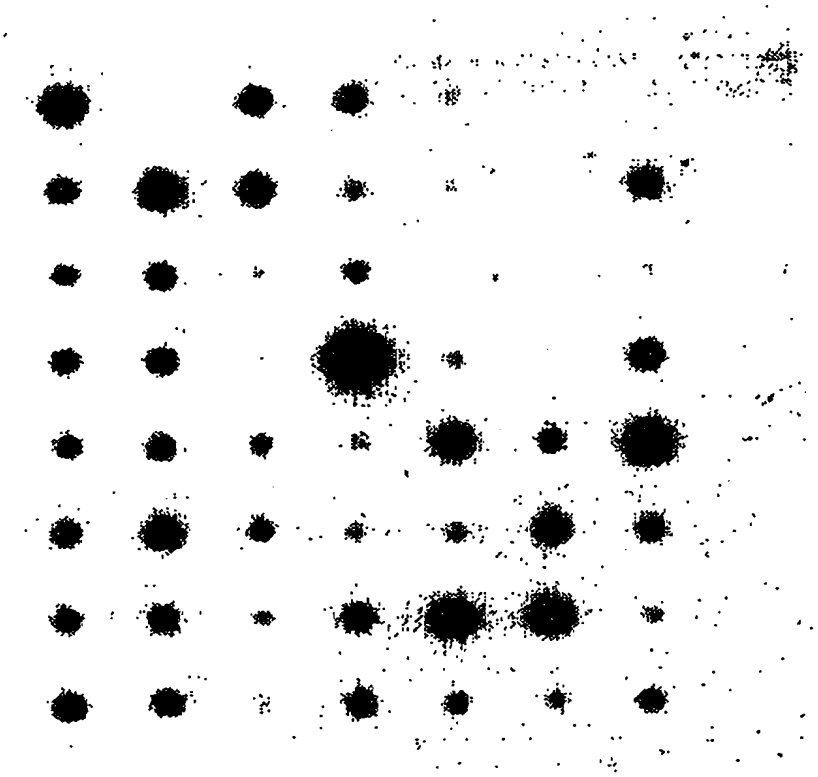


Figure 6

Figure 7

Expression of Seladin-1 in rat brain

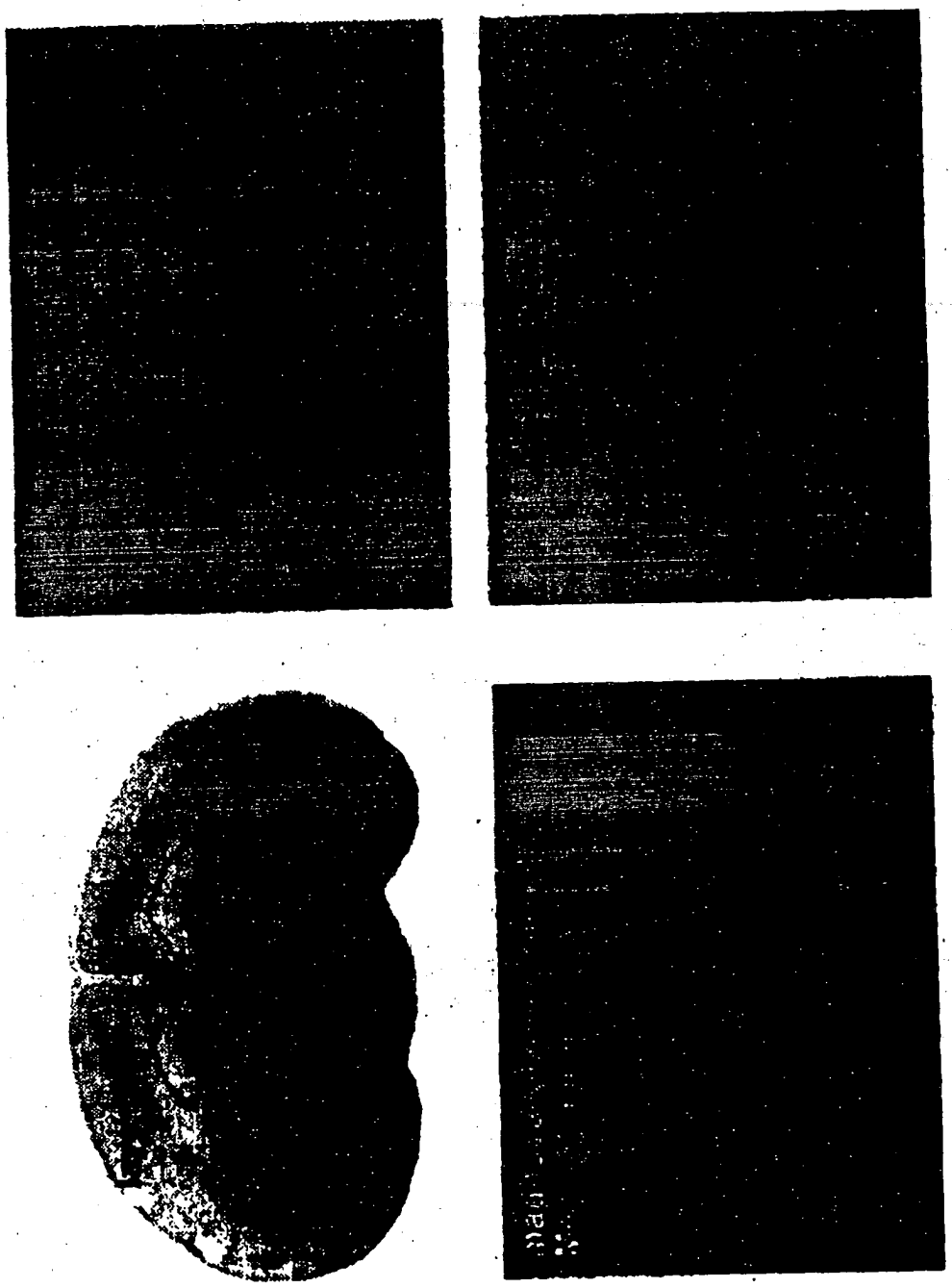


Figure 8

Expression of Seladin-1 in rat brain

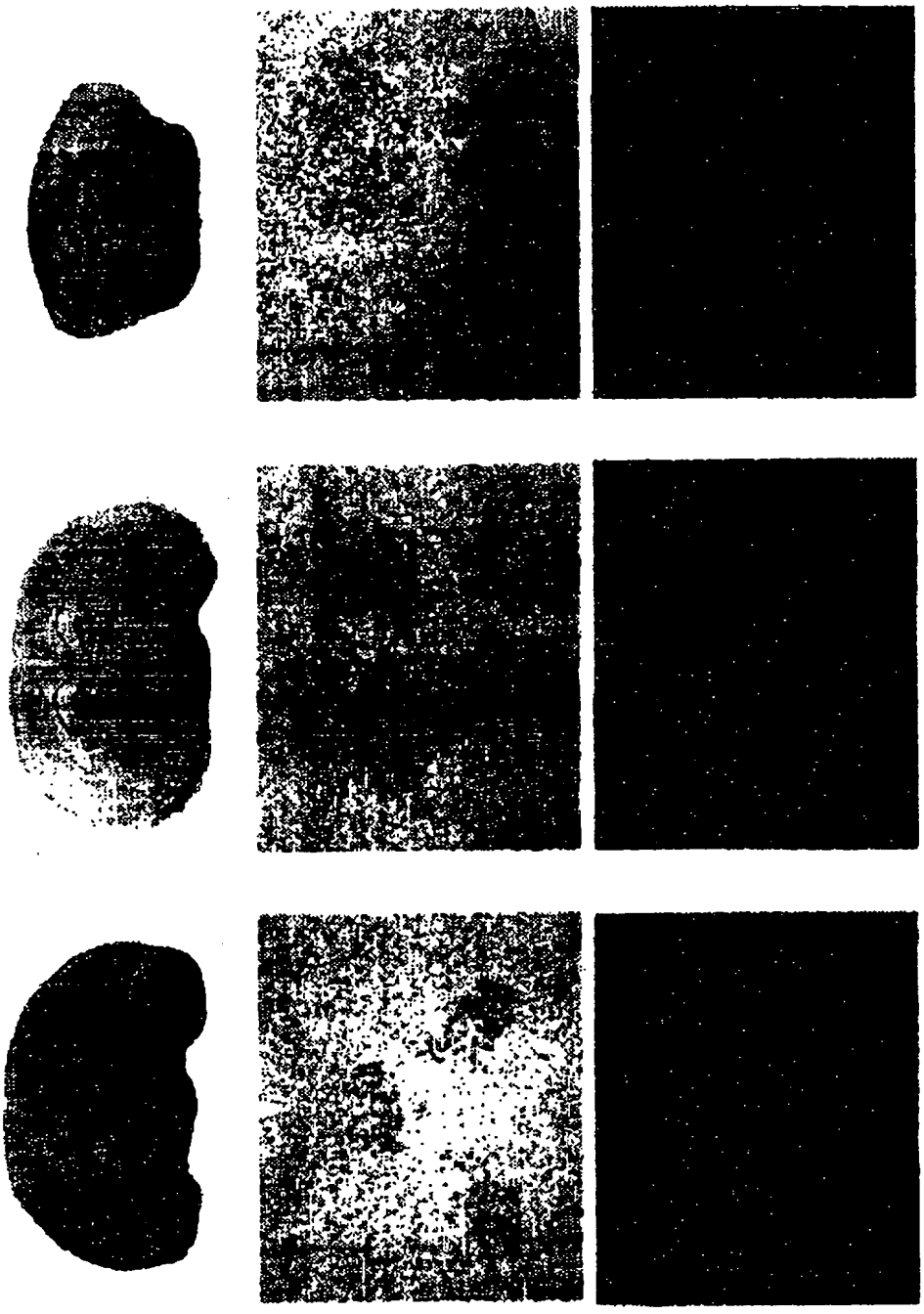


Figure 9

Expression of Seladin-1 in rat brain

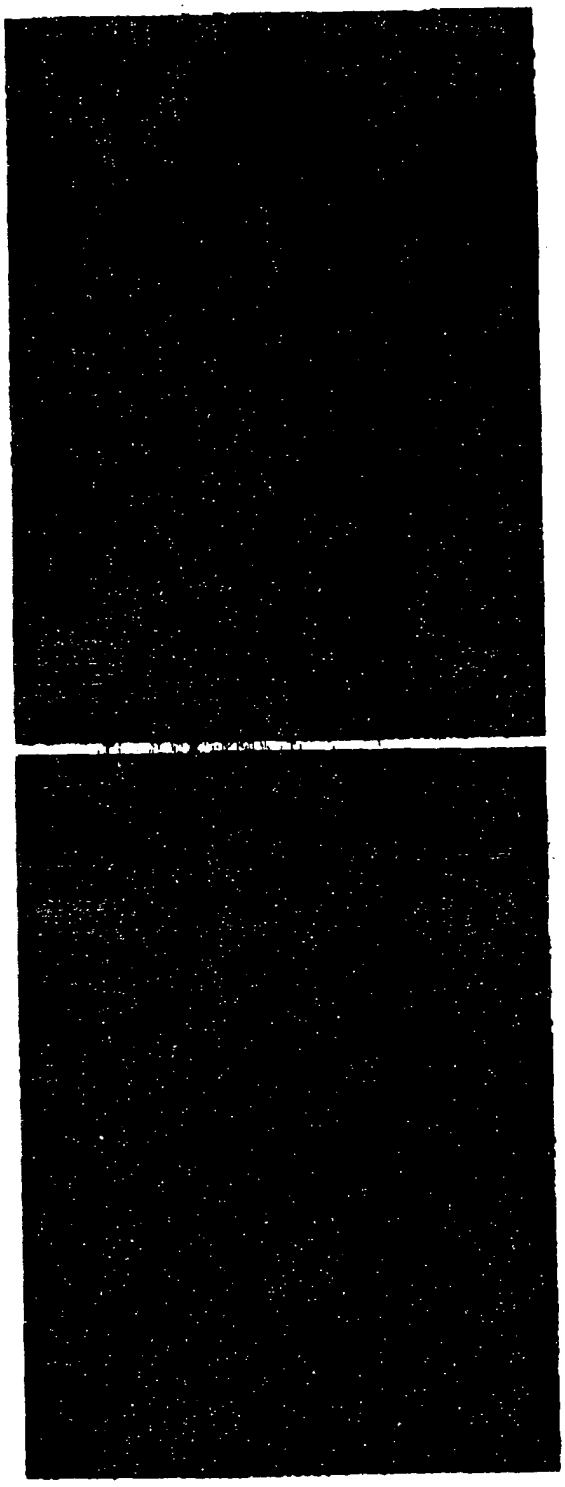
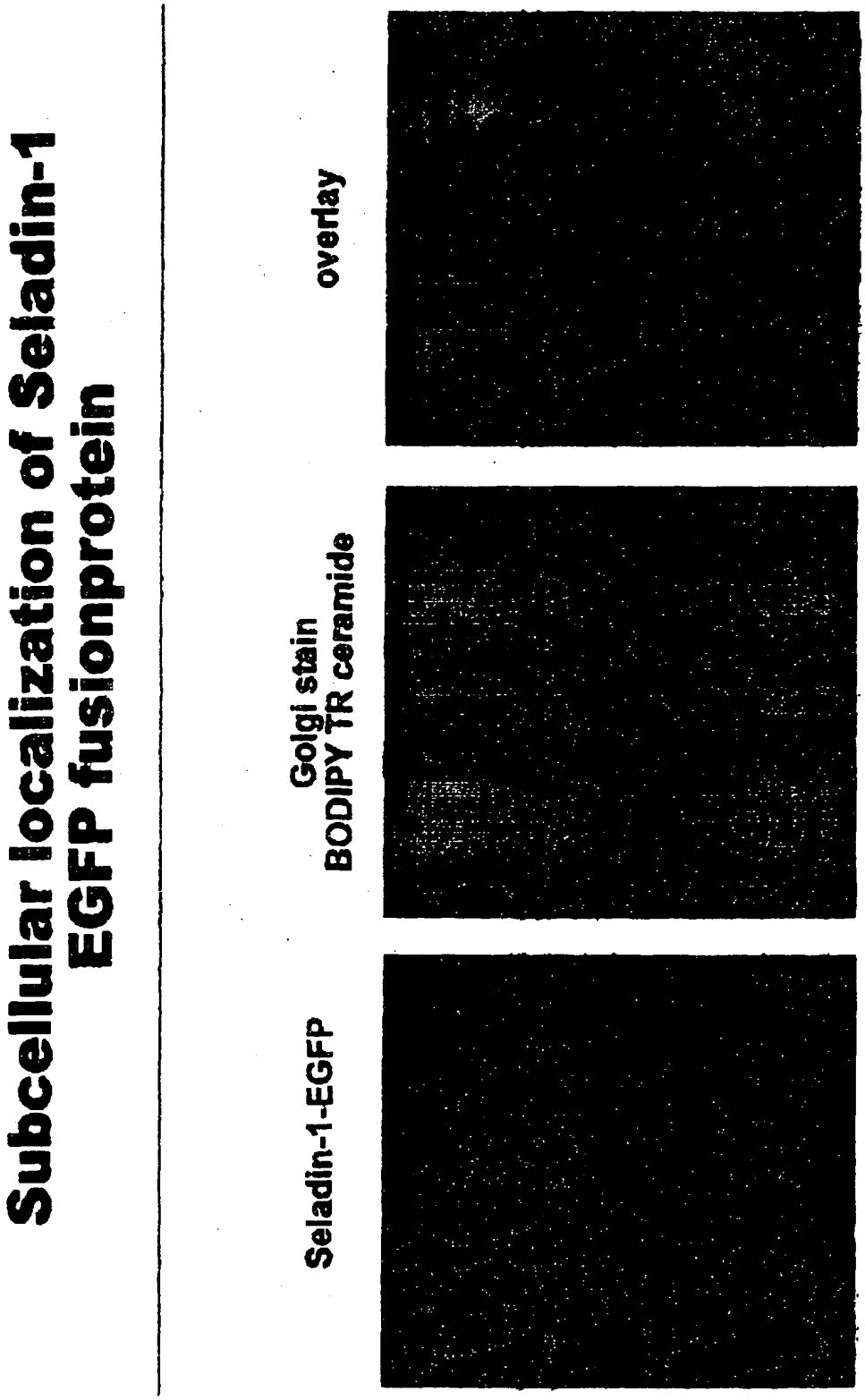


Figure 10


Subcellular localization of Seladin-1
EGFP fusionprotein
Seladin-1-EGFP
Golgi stain
BODIPY TR ceramide
overlay

Subcellular localization of Seladin-1 EGFP fusionprotein

Figure 11

12-11-1998 ENCHEN 06
-11-98
EP9812147866
+49 89 23994
SPEC

Printed 11-01-2000

12-11-1998 06:50 MUENCHEN 06 +49 89 23994465 SPEC
12-11-98 ; EP9812147B6 221 184297+

Multiple sequence alignments and secondary structure prediction of Seladin-1

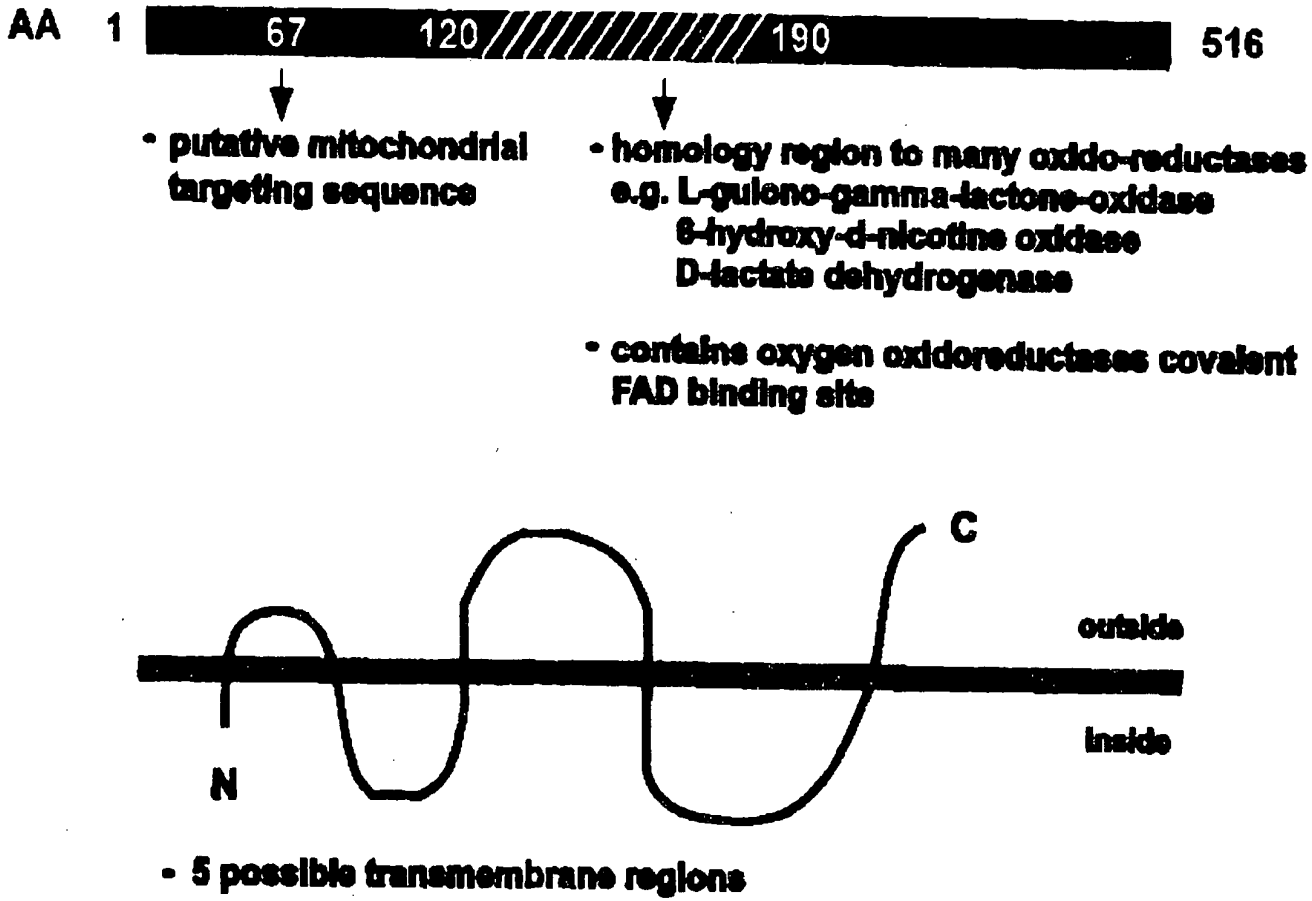


Figure 12

FIGURE 13: SEQ ID NO.1

Seladin-1 amino acid sequence

Seladin-1.orf Length: 516 May 29, 1998 14:51 Type: P Check: 1354 ..

```
1    MEPAVSLAVC ALLFLLWVRL KGLEFVLIHQ RWVFVCLFLL PLSLIFDIYY
51   YVRAWVVFKL SSAPRLHEQR VRDIQKQVRE WKEQGSKTFM CTGRPGWLTV
101  SLRVGKYKKT HKNIMINLMD ILEVDTKKQI VRVEPLVTMG QVTALLTSIG
151  WTLPVLPELD DLTVGGLIMG TGIESSSHKY GLFQHICTAY ELVLADGSFV
201  RCTPSENSDL FYAVPWSCGT LGFLVAAEIR IIPAKKYVKL RFEPVRGLEA
251  ICAKFTHESQ RQENHFVEGL LYSLDEAVIM TGVMTDEAEP SKLNSIGNYY
301  KPWFFKHVEN YLKTNREGLE YIPLRHYYHR HTRSIFWELQ DIIPFGNNPI
351  FRYLFGWMVP PKISLLKLTQ GETLRKLYEQ HHVVQDMLVP MKCLQQALHT
401  FQNDIHVYPI WLCPFILPSQ PGLVHPKGNE AELYIDIGAY GEPRVKHFEA
451  RSCMRQLEKF VRSVHGFQML YADCYMNREE FWEMFDGSLY HKLREKLGCQ
501  DAFPEVYDKI CKAARH
```

FIGURE 14: SEQ ID NO.2

Seladin-1 cDNA sequence

Seladin-1 Length: 4248 April 28, 1998 14:10 Type: N Check: 8184 ..

```
   1 cccgggctgt gggctacagg cgcagagcgg gccaggcgcg gagctggcgg
  51 cagtgacagg aggcgcgaac ccgcagcgct taccgcgcgg cgccgcacca
 101 tggagcccgc cgtgtcgctg gccgtgtgcg cgctgctctt cctgctgtgg
 151 gtgcgcctga aggggctgga gttcgtgctc atccaccagc gctgggtgtt
 201 cgtgtgcctc ttcctcctgc cgctctcgct tatcttcgat atctactact
 251 acgtgcgcgc ctgggtggtg ttcaagctca gcagcgctcc gcgcctgcac
 301 gagcagcgcg tgcgggacat ccagaagcag gtgcgggaat ggaaggagca
 351 gggtagcaag accttcatgt gcacggggcg ccctggctgg ctcactgtct
 401 cactacgtgt cgggaagtac aagaagacac acaaaaacat catgatcaac
 451 ctgatggaca ttctggaagt ggacaccaag aaacagattg tccgtgtgga
 501 gcccttggtg accatgggcc aggtgactgc cctgctgacc tccattggct
 551 ggactctccc cgtgttgcct gagcttgatg acctcacagt gggggggcttg
 601 atcatgggca caggcatcga gtcatcatcc cacaagtacg gcctgttcca
 651 acacatctgc actgcttacg agctggtcct ggctgatggc agctttgtgc
 701 gatgcactcc gtccgaaaac tcagacctgt tctatgccgt accctggtcc
 751 tgtgggacgc tgggtttcct ggtggccgct gagatccgca tcatccctgc
 801 caagaagtac gtcaagctgc gtttcgagcc agtgcggggc ctggaggcta
 851 tctgtgccaa gttcacccac gagtcccagc ggcaggagaa ccacttcgtg
 901 gaagggctgc tctactccct ggatgaggct gtcattatga caggggtcat
 951 gacagatgag gcagagccca gcaagctgaa tagcattggc aattactaca
1001 agccgtggtt ctttaagcat gtggagaact atctgaagac aaaccgagag
1051 ggcctggagt acattccctt gagacactac taccaccgcc acacgcgcag
1101 catcttctgg gagctccagg acatcatccc ctttggcaac aaccccatct
1151 tccgctacct ctttggctgg atggtgcctc ccaagatctc cctcctgaag
1201 ctgacccagg gtgagaccct gcgcaagctg tacgagcagc accacgtggt
1251 gcaggacatg ctggtgccca tgaagtgcct gcagcaggcc ctgcacacct
1301 tccaaaacga catccacgtc taccccatct ggctgtgtcc gttcatcctg
1351 cccagccagc caggcctagt gcaccccaaa ggaaatgagg cagagctcta
1401 catcgac tt ggagcatatg gggagccgcg tgtgaaacac tttgaagcca
1451 ggtcctgcat gaggcagctg gagaagtttg tccgcagcgt gcatggcttc
1501 cagatgctgt atgccgactg ctacatgaac cgggaggagt tctgggagat
1551 gtttgatggc tccttgtacc acaagctgcg agagaagctg ggttgccagg
```

```
1601  acgccttccc cgaggtgtac gacaagatct gcaaggccgc caggcactga
1651  gctggagccc gcctggagag acagacacgt gtgagtggtc aggcatcttc
1701  ccttcactca agcttggctg ctttcctaga tccacacttt caaagagaaa
1751  cccctccaga actcccaccc tgacagccca acaccacctt cctcctggct
1801  tccagggggc agcccagtgg aatggaaaga atgtgggatt tggagtcaga
1851  caagcctgag tccagttccc cgtttagaac tcattagctg tgtgactctg
1901  ggtgagtccc ttaacccctc tgagcccggg tctcttcatt agttgaaagg
1951  gatagtaata cctacttgca ggttgttgtc atctgagttg agcactggtc
2001  acattgaagg tgctgggtaa gtggtagctc ttgttgcttc ccgttcagcg
2051  tcacatctgc agtggagcct gaaaaggctc cacattaggt cacctgtgca
2101  cagccatggc tggaatgatg aaggggatac gctggagttg ccctgccatc
2151  gcctccatca gccagacgag gtcctcacag gagaaggaca gctcttcccc
2201  accctgggat ctcaggaggg cagccacgga gtggggaggc cccagatgcg
2251  ctgtgccaaa gccaggtccg aggccaaagt tctccctgcc atccttggtg
2301  ccgtcctgcc ccttcctcct tcatgcctgg gcctgcaggc ccaccccagc
2351  caccactgag tccactcgga gtgccctgtg ttcctggaga aggcattcca
2401  gggttgaatc ttgtcccagc ctcagcctgg gacacctagg tggagagagt
2451  ggtctccgct ctgaattgga tccaggggac ctgggctcat tcttcttggc
2501  tcaccaaccc tgcaggcctc atctttccca aaacccactt tgtcttggtg
2551  ggagtgggtc cgcgctgctc tgcagcaggg gctggggagt ggacagcatc
2601  aggtgggaaa gtggagtcca cccicatgtt tctgtaggat tctcaccgtg
2651  gggctggaag aaaagagcat cgacttgatt tctccaacca ctcatcccic
2701  tttttctttc ttccaccact ccccacccca gctgtagtta atttcagtgc
2751  cttacaaatc ctaagctcag agaaagttcc atttccgttc cagagggaag
2801  ggaacctccc taggtccttc cctggcttgt tataacgcaa agcttggttg
2851  tttatgcaac tctatcttaa gaactgccca gcctcagctg aaaacccgaa
2901  tctgagaagg aattgcgtca tgtaagggaa gctggaatta agggagctga
2951  gccagtcatg gttgtggcgt gtgagtcagg agacctaggt ttcagccccc
3001  ctctactgtc agcgagctgt gcaacgtggg caagtcattg tcctctgagc
3051  tgcagtttcc tcatctgtca catcgctaca gacaagacct ccctggaacc
3101  cttctgattg tcttagacac tgtggttgca aaacccacgg aaagcctcat
3151  ttgtgtggaa agtcagagga aaaatgatcc agtggacact tggggattat
3201  ctgtcattca agatccttcc ttcaacccca aggccagctc ccatctcatt
3251  tccagaaagg ctcatacctg gcttgcaggg aagcatctgt cttgtcattc
3301  caggtgccag aatcctctca gagtcattga agggtgttca cccatcccac
```

```
3351  ccaaggcttg gcacactgcc agtgtcttag cagggtcttg tgagggctgg
3401  gggcatccag gcactcagaa ggcaaaggaa ccaccctacc catttggcct
3451  ctggaggggg cagaagaaag aaagaaacct catcctatat tttacaaagc
3501  atgtgaattc tggcattagc tctcatagga gacccatgtg cttccttgct
3551  cagtgcaaaa ctgatgattc tacttgctgt agatgaatgg ttaacacgag
3601  ctagttaaac agtgccattg ttttgccagt gaagcctcca accctaagcc
3651  actgggacgg tggccagaga tgccagcagc ctctgtcgcc cttagtcata
3701  taaccaaaat ccagacctta tccacaaccc ggggcttgga aaggaaggta
3751  ttttggaatc acaccctccg gttatgttgc tccagtaaaa tcttgcctgg
3801  aaagaggcag tcttcttagc atggtgagct gagttcatgg ctttttttg
3851  tagccagtcc tgtccctggc catccatgtg atggttttgg atggagttaa
3901  acttgatgcc agtgggcagt gcatgtggaa agtatcagag taagcctctc
3951  ccctccagag ccctgagttt cttggctgca tgaaggtttt ctttagaatc
4001  agaattgtag ccagtttctt tggccagaag gatgaatact tggatattac
4051  tgaaagggag gggtggagat gggtgtggca gtgtatggtg tgtgattttt
4101  attttcttct ttggtcatgg gggccaagga gaaaggcatg aatcttccct
4151  gtcaggctct tacagccaca ggcactgtgt ctactgtctg gaagacatgt
4201  ccccgtggct gtggggccgc tgcttctgtt taaataaaag tggcctgg
```

FIGURE 15 CDNA sequence comparison KIAA0018/Seladin-1

```
  1 ggcgcgaacccgcagcgcttaccgcgcggcgccgcaccatgg gcccgcc 50
    ||||||||||||||||||||||||||||||||||||||||| |||||||
 62 ggcgcgaacccgcagcgcttaccgcgcggcgccgcaccatggagcccgcc 111

 51 gtgtcgctggccgtgtgcgcgctgctcttcctgctgtgggtgcgcctgaa 100
    ||||||||||||||||||||||||||||||||||||||||||||||||||
112 gtgtcgctggccgtgtgcgcgctgctcttcctgctgtgggtgcgcctgaa 161

101 ggggctggagttcgtgctcatccaccagcgctgggtgttcgtgtgcctct 150
    ||||||||||||||||||||||||||||||||||||||||||||||||||
162 ggggctggagttcgtgctcatccaccagcgctgggtgttcgtgtgcctct 211

151 tcctcctgccgctctcgcttatcttcgatatctactactacgtgcgcgcc 200
    ||||||||||||||||||||||||||||||||||||||||||||||||||
212 tcctcctgccgctctcgcttatcttcgatatctactactacgtgcgcgcc 261

201 tgggtggtgttcaagctcagcagcgctccgcgcctgcacgagcagcgcgt 250
    ||||||||||||||||||||||||||||||||||||||||||||||||||
262 tgggtggtgttcaagctcagcagcgctccgcgcctgcacgagcagcgcgt 311

251 gcgggacatccagaagcaggtgcgggaatggaaggagcagggtagcaaga 300
    ||||||||||||||||||||||||||||||||||||||||||||||||||
312 gcgggacatccagaagcaggtgcgggaatggaaggagcagggtagcaaga 361

301 ccttcatgtgcacggggcgccctggctggctcactgtctcactacgtgtc 350
    ||||||||||||||||||||||||||||||||||||||||||||||||||
362 ccttcatgtgcacggggcgccctggctggctcactgtctcactacgtgtc 411

351 gggaagtacaagaagacacacaaaaacatcatgatcaacctgatggacat 400
    ||||||||||||||||||||||||||||||||||||||||||||||||||
412 gggaagtacaagaagacacacaaaaacatcatgatcaacctgatggacat 461

401 tctggaagtggacaccaagaaacagattgtccgtgtggagcccttggtga 450
    ||||||||||||||||||||||||||||||||||||||||||||||||||
462 tctggaagtggacaccaagaaacagattgtccgtgtggagcccttggtga 511

451 ccatgggccaggtgactgccctgctgacctccattggctggactctcccc 500
    ||||||||||||||||||||||||||||||||||||||||||||||||||
512 ccatgggccaggtgactgccctgctgacctccattggctggactctcccc 561

501 gtgttgcctgagcttgatgacctcacagtgggggggcttgatcatgggcac 550
    ||||||||||||||||||||||||||||||||||||||||||||||||||
562 gtgttgcctgagcttgatgacctcacagtgggggggcttgatcatgggcac 611

551 aggcatcgagtcatcatcccacaagtacggcctgttccaacacatctgca 600
    ||||||||||||||||||||||||||||||||||||||||||||||||||
612 aggcatcgagtcatcatcccacaagtacggcctgttccaacacatctgca 661

601 ctgcttacgagctggtcctggctgatggcagctttgtgcgatgcactccg 650
    ||||||||||||||||||||||||||||||||||||||||||||||||||
662 ctgcttacgagctggtcctggctgatggcagctttgtgcgatgcactccg 711

651 tccgaaaactcagacctgttctatgccgtaccctggtcctgtgggacgct 700
    ||||||||||||||||||||||||||||||||||||||||||||||||||
712 tccgaaaactcagacctgttctatgccgtaccctggtcctgtgggacgct 761

701 gggtttcctggtggccgctgagatccgcatcatccctgccaagaagtacg 750
    ||||||||||||||||||||||||||||||||||||||||||||||||||
762 gggtttcctggtggccgctgagatccgcatcatccctgccaagaagtacg 811

751 tcaagctgcgtttcgagccagtgcggggcctggaggctatctgtgccaag 800
    ||||||||||||||||||||||||||||||||||||||||||||||||||
812 tcaagctgcgtttcgagccagtgcggggcctggaggctatctgtgccaag 861

801 ttcacccacgagtcccagcggcaggagaaccacttcgtggaagggctgct 850
    ||||||||||||||||||||||||||||||||||||||||||||||||||
862 ttcacccacgagtcccagcggcaggagaaccacttcgtggaagggctgct 911
```

```
851 ctactccctggatgaggctgtcattatgacaggggtcatgacagatgagg 900
    ||||||||||||||||||||||||||||||||||||||||||||||||||||
912 ctactccctggatgaggctgtcattatgacaggggtcatgacagatgagg 961

901 cagagcccagcaagctgaatagcattggcaattactacaagccgtggttc 950
    ||||||||||||||||||||||||||||||||||||||||||||||||||||
962 cagagcccagcaagctgaatagcattggcaattactacaagccgtggttc 1011

951 tttaagcatgtggagaactatctgaagacaaaccgagagggcctggagta 1000
    ||||||||||||||||||||||||||||||||||||||||||||||||||||
1012 tttaagcatgtggagaactatctgaagacaaaccgagagggcctggagta 1061

1001 cattcccttgagacactactaccaccgccacacgcgcagcatcttctggg 1050
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1062 cattcccttgagacactactaccaccgccacacgcgcagcatcttctggg 1111

1051 agctccaggacatcatcccctttggcaacaaccccatcttccgctacctc 1100
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1112 agctccaggacatcatcccctttggcaacaaccccatcttccgctacctc 1161

1101 tttggctggatggtgcctcccaagatctccctcctgaagctgacccaggg 1150
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1162 tttggctggatggtgcctcccaagatctccctcctgaagctgacccaggg 1211

1151 tgagaccctgcgcaag.tgtacgagcagcaccacgtggtgcaggacatgc 1199
     |||||||||||||||| ||||||||||||||||||||||||||||||||||
1212 tgagaccctgcgcaagctgtacgagcagcaccacgtggtgcaggacatgc 1261

1200 tggtgcccatgaagtgcctgcagcaggccctgcacaccttccaaaacgac 1249
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1262 tggtgcccatgaagtgcctgcagcaggccctgcacaccttccaaaacgac 1311

1250 atccacgtctaccccatctggctgtgtccgttcatcctgcccagccagcc 1299
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1312 atccacgtctaccccatctggctgtgtccgttcatcctgcccagccagcc 1361

1300 aggcctagtgcaccccaaaggaaatgaggcagagctctacatcgacattg 1349
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1362 aggcctagtgcaccccaaaggaaatgaggcagagctctacatcgacattg 1411

1350 gagcatatggggagccgcgtgtgaaacactttgaagccaggtcctgcatg 1399
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1412 gagcatatggggagccgcgtgtgaaacactttgaagccaggtcctgcatg 1461

1400 aggcagctggagaagtttgtccgcagcgtgcatggcttccagatgctgta 1449
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1462 aggcagctggagaagtttgtccgcagcgtgcatggcttccagatgctgta 1511

1450 tgccgactgctacatgaaccgggaggagttctgggagatgtttgatggct 1499
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1512 tgccgactgctacatgaaccgggaggagttctgggagatgtttgatggct 1561

1500 ccttgtaccacaagctgcgagagaagctgggttgccaggacgccttcccc 1549
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1562 ccttgtaccacaagctgcgagagaagctgggttgccaggacgccttcccc 1611

1550 gaggtgtacgacaagatctgcaaggccgccaggcactgagctggagcccg 1599
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1612 gaggtgtacgacaagatctgcaaggccgccaggcactgagctggagcccg 1661

1600 cctggagagacagacacgtgtgagtggtcaggcatcttcccttcactcaa 1649
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1662 cctggagagacagacacgtgtgagtggtcaggcatcttcccttcactcaa 1711

1650 gcttggctgctttcctagatccacactttcaaagagaaaccccctccagaa 1699
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1712 gcttggctgctttcctagatccacactttcaaagagaaaccccctccagaa 1761
```

```
1700 ctcccaccctgacagcccaacaccaccttcctcctggcttccaggggca 1749
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1762 ctcccaccctgacagcccaacaccaccttcctcctggcttccaggggca 1811

1750 gcccagtggaatggaaagaatgtgggatttggagtcagacaagcctgagt 1799
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1812 gcccagtggaatggaaagaatgtgggatttggagtcagacaagcctgagt 1861

1800 ccagttccccgtttagaactcattagctgtgtgactctgggtgagtccct 1849
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1862 ccagttccccgtttagaactcattagctgtgtgactctgggtgagtccct 1911

1850 taacccctctgagcccgggtctcttcattagttgaaagggatagtaatac 1899
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1912 taacccctctgagcccgggtctcttcattagttgaaagggatagtaatac 1961

1900 ctacttgcaggttgttgtcatctgagttgagcactggtcacattgaaggt 1949
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1962 ctacttgcaggttgttgtcatctgagttgagcactggtcacattgaaggt 2011

1950 gctgggtaagtggtagctcttgttgcttcccgttcagcgtcacatctgca 1999
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2012 gctgggtaagtggtagctcttgttgcttcccgttcagcgtcacatctgca 2061

2000 gtggagcctgaaaaggctccacattaggtcacctgtgcacagccatggct 2049
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2062 gtggagcctgaaaaggctccacattaggtcacctgtgcacagccatggct 2111

2050 ggaatgatgaaggggatacgctggagttgccctgccatcgcctccatcag 2099
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2112 ggaatgatgaaggggatacgctggagttgccctgccatcgcctccatcag 2161

2100 ccagacgaggtcctcacaggagaaggacagctcttccccaccctgggatc 2149
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2162 ccagacgaggtcctcacaggagaaggacagctcttccccaccctgggatc 2211

2150 tcaggagggcagccacggagtggggaggccccagatgcgctgtgccaaag 2199
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2212 tcaggagggcagccacggagtggggaggccccagatgcgctgtgccaaag 2261

2200 ccaggtccgaggccaaagttctccctgccatccttggtgccgtcctgccc 2249
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2262 ccaggtccgaggccaaagttctccctgccatccttggtgccgtcctgccc 2311

2250 cttcctccttcatgcctgggcctgcaggcccaccccagccaccactgagt 2299
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2312 cttcctccttcatgcctgggcctgcaggcccaccccagccaccactgagt 2361

2300 ccactcggagtgccctgtgttcctggagaaggcattccagggttgaatct 2349
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2362 ccactcggagtgccctgtgttcctggagaaggcattccagggttgaatct 2411

2350 tgtcccagcctcagcctgggacacctaggtggagagagtggtctccgctc 2399
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2412 tgtcccagcctcagcctgggacacctaggtggagagagtggtctccgctc 2461

2400 tgaattggatccaggggacctgggctcattcttcttggctcaccaaccct 2449
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2462 tgaattggatccaggggacctgggctcattcttcttggctcaccaaccct 2511

2450 gcaggcctcatcttttcccaaaacccactttgtcttggtgggagtgggtcc 2499
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2512 gcaggcctcatcttttcccaaaacccactttgtcttggtgggagtgggtcc 2561

2500 gcgctgctctgcagcaggggctggggagtggacagcatcaggtgggaaag 2549
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2562 gcgctgctctgcagcaggggctggggagtggacagcatcaggtgggaaag 2611
```

```
2550 tggagtccaccctcatgtttctgtaggattctcaccgtggggctggaaga 2599
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2612 tggagtccaccctcatgtttctgtaggattctcaccgtggggctggaaga 2661

2600 aaagagcatcgacttgatttctccaaccactcatccctcttttttctttct 2649
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2662 aaagagcatcgacttgatttctccaaccactcatccctcttttttctttct 2711

2650 tccaccactccccaccccagctgtagttaatttcagtgccttacaaatcc 2699
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2712 tccaccactccccaccccagctgtagttaatttcagtgccttacaaatcc 2761

2700 taagctcagagaaagttccatttccgttccagagggaagggaacctccct 2749
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2762 taagctcagagaaagttccatttccgttccagagggaagggaacctccct 2811

2750 aggtccttccctggcttgttataacgcaaagcttggttgtttatgcaact 2799
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2812 aggtccttccctggcttgttataacgcaaagcttggttgtttatgcaact 2861

2800 ctatcttaagaactgcccagcctcagctgaaaacccgaatctgagaagga 2849
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2862 ctatcttaagaactgcccagcctcagctgaaaacccgaatctgagaagga 2911

2850 attgcgtcatgtaagggaagctggaattaagggagctgagccagtcatgg 2899
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2912 attgcgtcatgtaagggaagctggaattaagggagctgagccagtcatgg 2961

2900 ttgtggcgtgtgagtcaggagacctaggtttcagcccctctctactgtca 2949
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2962 ttgtggcgtgtgagtcaggagacctaggtttcagcccctctctactgtca 3011

2950 gcgagctgtgcaacgtgggcaagtcattgtcctctgagctgcagtttcct 2999
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3012 gcgagctgtgcaacgtgggcaagtcattgtcctctgagctgcagtttcct 3061

3000 catctgtcacatcgctacagacaagacctccctggaacccttctgattgt 3049
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3062 catctgtcacatcgctacagacaagacctccctggaacccttctgattgt 3111

3050 cttagacactgtggttgcaaaacccacggaaagcctcatttgtgtggaaa 3099
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3112 cttagacactgtggttgcaaaacccacggaaagcctcatttgtgtggaaa 3161

3100 gtcagaggaaaaatgatccagtggacacttggggattatctgtcattcaa 3149
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3162 gtcagaggaaaaatgatccagtggacacttggggattatctgtcattcaa 3211

3150 gatccttccttcaaccccaaggccagctcccatctcatttccagaaaggc 3199
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3212 gatccttccttcaaccccaaggccagctcccatctcatttccagaaaggc 3261

3200 tcatacctggcttgcagggaagcatctgtcttgtcattccaggtgccaga 3249
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3262 tcatacctggcttgcagggaagcatctgtcttgtcattccaggtgccaga 3311

3250 atcctctcagagtcattgaagggtgttcacccatcccacccaaggcttgg 3299
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3312 atcctctcagagtcattgaagggtgttcacccatcccacccaaggcttgg 3361

3300 cacactgccagtgtcttagcagggtcttgtgagggctgggggcatccagg 3349
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3362 cacactgccagtgtcttagcagggtcttgtgagggctgggggcatccagg 3411

3350 cactcagaaggcaaaggaaccaccctacccatttggcctctggaggggggc 3399
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3412 cactcagaaggcaaaggaaccaccctacccatttggcctctggaggggggc 3461
```

```
3400 agaagaaagaaagaaacctcatcctatattttacaaagcatgtgaattct 3449
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3462 ag agaaagaaagaaacctcatcctatattttacaaagcatgtgaattct 3511

3450 ggcattagctctcataggagacccatgtgcttccttgctcagtgcaaaac 3499
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3512 ggcattagctctcataggagacccatgtgcttccttgctcagtgcaaaac 3561

3500 tgatgattctacttgctgtagatgaatggttaacacgagctagttaaaca 3549
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3562 tgatgattctacttgctgtagatgaatggttaacacgagctagttaaaca 3611

3550 gtgccattgttttgccagtgaagcctccaaccctaagccactgggacggt 3599
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3612 gtgccattgttttgccagtgaagcctccaaccctaagccactgggacggt 3661

3600 ggccagagatgccagcagcctctgtcgcccttagtcatataaccaaaatc 3649
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3662 ggccagagatgccagcagcctctgtcgcccttagtcatataaccaaaatc 3711

3650 cagaccttatccacaacccggggcttggaaaggaaggtattttggaatca 3699
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3712 cagaccttatccacaacccggggcttggaaaggaaggtattttggaatca 3761

3700 caccctccggttatgttgctccagtaaaatcttgcctggaaagaggcagt 3749
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3762 caccctccggttatgttgctccagtaaaatcttgcctggaaagaggcagt 3811

3750 cttcttagcatggtgagctgagttcatggctttttttttgtagccagtcct 3799
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3812 cttcttagcatggtgagctgagttcatggctttttttttgtagccagtcct 3861

3800 gtcccctggccatccatgtgatggttttggatggagttaaacttgatgcca 3849
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3862 gtcccctggccatccatgtgatggttttggatggagttaaacttgatgcca 3911

3850 gtgggcagtgcatgtggaaagtatcagagtaagcctctcccctccagagc 3899
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3912 gtgggcagtgcatgtggaaagtatcagagtaagcctctcccctccagagc 3961

3900 cctgagtttcttggctgcatgaaggttttctttagaatcagaattgtagc 3949
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3962 cctgagtttcttggctgcatgaaggttttctttagaatcagaattgtagc 4011

3950 cagtttctttggccagaaggatgaatacttggatattactgaaagggagg 3999
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
4012 cagtttctttggccagaaggatgaatacttggatattactgaaagggagg 4061

4000 ggtggagatgggtgtggcagtgtatggtgtgtgatttttatttttcttctt 4049
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
4062 ggtggagatgggtgtggcagtgtatggtgtgtgatttttatttttcttctt 4111

4050 tggtcatgggggccaaggagaaaggcatgaatcttcccctgtcaggctctt 4099
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
4112 tggtcatgggggccaaggagaaaggcatgaatcttcccctgtcaggctctt 4161

4100 acagccacaggcactgtgtctactgtctggaagacatgtccccgtggctg 4149
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
4162 acagccacaggcactgtgtctactgtctggaagacatgtccccgtggctg 4211

4150 tggggccgctgcttctgtttaaataaaagtggcctgg 4186
     ||||||||||||||||||||||||||||||||||||
4212 tggggccgctgcttctgtttaaataaaagtggcctgg 4248
```

Abstract

An isolated nucleic acid molecule encoding a protein molecule shown in SEQ ID No. 1.

THIS PAGE BLANK (USPTO)